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(71)Applicant : JAPAN TOBACCO INC
(72)Inventor : TSUJI TAKASHI
WATABE YOSHIHIRO
WAGA IWAO

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(54) PRODUCTION OF HEMATOPOIETIC STEM CELL

(57)Abstract:
PROBLEM TO BE SOLVED: To readily and efficiently produce CD34-positive cells excellent in safety and useful for treatment of acute leukemia, tumorous disease, etc., in a short time in a high yield, by culturing human CD34-positive cells in a nutrient medium containing stroma cells derived from a mammal.
SOLUTION: Human CD34-positive cells such as human CD34-strongly positive cells derived from umbilical cord blood, spinal fluid, liver, spleen and peripheral blood is cultured in a nutrient medium having abilities capable of propagating human CD34-positive cells, derived from a mammal such as mouse, containing stroma cells such as HESS-1 and HESS-5 (FERM BP-5768), and preferably containing cytokine such as interleukin-3 and hepatic cell factor to propagate the human CD34-positive cells and to provide the human CD34-positive cells in the method for producing the human CD34-positive cells.

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CLAIMS

Claim(s)]
Claim 1] The manufacture approach of CD34 positivity cell of the Homo sapiens characterized by proliferating his Homo sapiens's CD34 positivity cell by cultivating in the nutrition culture medium containing the stromata cell of the mammalian origin which has the capacity to proliferate human CD34 positivity cell for human CD34 positivity cell.
Claim 2] The manufacture approach according to claim 1 that CD34 positivity cell manufactured by culture is characterized by being 34 or so CD positivity cell.
Claim 3] The manufacture approach according to claim 1 or 2 characterized by CD34 positivity cell being CD34 positivity cell originating in cord blood, bone marrow, liver, a spleen, or peripheral blood.
Claim 4] The manufacture approach according to claim 1 to 3 characterized by being the organization of a mouse which a stromata cell includes for the stromata cell or this stromata cell of the mouse origin.
Claim 5] A stromata cell HESS-1, HESS-5 (international deposition number: FERM BP-5768), HESS-18 international deposition number: FERM BP-6187), HESS-M28 (international deposition number: FERM BP-6186), That they are one sort or two sorts or more of stromata cells chosen from the group which consists of a stromata cell of the mouse origin respectively named SSXL CL.1, SSXL CL3, SSXL CL.7, SSXL CL.9, and SSXL CL.17 The manufacture approach according to claim 4 by which it is characterized.
Claim 6] The manufacture approach according to claim 4 characterized by being the stromata cell chosen from the group which a stromata cell becomes from the stromata cell strain of the mouse origin respectively named HESS-5 (international deposition number: FERM BP-5768), HESS-18 (international deposition number: FERM BP-6187), HESS-M28 (international deposition number: FERM BP-6186), and SSXL CL.3.
Claim 7] The manufacture approach according to claim 1 to 6 characterized by cultivating under existence of cytokine.
Claim 8] The manufacture approach according to claim 7 characterized by cytokine being one sort or two sorts or more of cytokine chosen from the group which consists of interleukin 3, stem cell factor (SCF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), flk2/flt3-ligand, and macrophage origin inflammatory protein 1alpha (MIP-1alpha) and erythropoietin (EPO).
Claim 9] The manufacture approach according to claim 1 to 8 that CD34 positivity cell is characterized by being cultivated in the state of a stromata cell, a contact condition, a non-contact condition, or indirect contact.
Claim 10] The manufacture approach according to claim 1 to 8 that CD34 positivity cell is characterized by being cultivated in the state of a stromata cell and indirect contact.
Claim 11] Human CD34 positivity cell obtained by the manufacture approach according to claim 1 to 10.
Claim 12] The physic constituent which consists of a CD34 positivity cell of the Homo sapiens obtained by the manufacture approach according to claim 1 to 10, and support which may be permitted by the pharmaceutical-sciences target.
Claim 13] The stromata cell strain of the mouse origin identified by international deposition number FERM BP-6187.
Claim 14] The stromata cell strain of the mouse origin identified by international deposition number FERM BP-6186.
Claim 15] It is a culture instrument for carrying out growth culture of the CD34 positivity cell which is the culture instrument which consists of at least one base material for supporting the cell held in the culture container and this culture container, and is characterized by for this base material to consist of support for making the supporting lamella and this supporting lamella for carrying out seeding support of either [at least]

CD34 positivity cell or a stromata cell into a culture medium fix to a container.
Claim 16] The 1st film which it is [film] an instrument for cultivating a cell, and the instrument:(a) nutrition culture medium and cytokine which are characterized by consisting of following members at least can be penetrated [film], and cannot pass a cell;
b) the 2nd film (here -- this -- two film) which it can be arranged [film] at the top-face side of this 1st film, can make a carbon dioxide able to penetrate, and cannot make a liquid penetrate the following -- opening by tubing of (d) being arranged -- removing -- this -- it is arranged so that the electric shielding system which has content volume which a liquid does not leak besides this instrument may be formed between two film. ;
c) the 3rd film (here -- this -- two film) which it can be arranged [film] at the inferior-surface-of-tongue side of this 1st film, can make a carbon dioxide able to penetrate, and cannot make a liquid penetrate the following -- opening by tubing of (e) being arranged -- removing -- this -- it is arranged so that the electric shielding system which has content volume which a liquid does not leak besides this instrument may be formed between two film. ;
d) Tubing of the 2nd ** which it is arranged [**] between this 1st film and the 2nd film, is arranged [**] between 1st tubing; which can make a liquid, a nutrition culture medium, and a cell pour in or discharge and the
e) this 1st film, and the 3rd film, and can make a liquid, a nutrition culture medium, and a cell pour in or discharge.
Claim 17] either of the electric shielding systems which have the content volume formed between the 1st film and the 2nd film or between the 1st film and the 3rd film -- this -- the instrument according to claim 16 characterized by being formed by arranging one side of two film at one side of an insoluble frame, and arranging the film of another side in other fields of this frame.
Claim 18] The instrument according to claim 16 or 17 characterized by including the nutrition culture medium in each of the electric shielding system which has the content volume formed between the 1st film and the 2nd film and between the 1st film and the 3rd film, and the cell having pasted one field of the 1st film.
Claim 19] The instrument according to claim 18 with which a cell is characterized by being the stromata cell of mammalian.
Claim 20] The instrument according to claim 16 or 17 characterized by containing the stromata cell of mammalian in either of the electric shielding systems which have the content volume formed between the 1st film and the 2nd film or between the 1st film and the 3rd film.
Claim 21] The instrument according to claim 19 or 20 characterized by a stromata cell being a stromata cell which has the capacity to proliferate human CD34 positivity cell.
Claim 22] The instrument according to claim 21 characterized by being the stromata cell chosen from the group which a stromata cell becomes from the stromata cell strain of the mouse origin respectively named HESS-5 (international deposition number: FERM BP-5768), HESS-18 (international deposition number: FERM BP-6187), HESS-M28 (international deposition number: FERMBP-6186), and SSXL CL.3.
Claim 23] The manufacture approach of CD34 positivity cell of the Homo sapiens characterized by using an instrument according to claim 15 to 22.

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)ETAILED DESCRIPTION

Detailed Description of the Invention]
0001]
Field of the Invention] This invention relates to the manufacture approach of a Homo sapiens CD34 positivity :ell of having used this culture instrument for the manufacture approach of human CD34 positivity cell, the cell :ulture instrument, and the list. CD34 positivity cell which contains a multipotential stem cell and/or HPP-CFC 'or various cytokine in content or a non-containing nutrition culture medium is proliferated under coexistence vith a stromata cell in more detail, and it is related with the approach of manufacturing, and the cell culture nstrument used for this manufacture at a list.
'0002]
[Description of the Prior Art] Each cell lineage train of lymphocytic series, such as a T cell, a B cell, etc. which :akes charge of myelocyte systems, such as erythron in connection with oxygen transport, a megakaryocyte ;ystem which produces a platelet, granulocyte in connection with the phylaxis, and monocyte/macrophage, and mmunity, is in blood as a corpuscle cell which manages a living body function. Any cell of a corpuscle cell is led ınd produced [maintain and] in the life span by specializing and maturing from the pluripotency hematopoietic ;tem cell which is the common origin. The pluripotency hematopoietic stem cell has the capacity (self-renewal ıbility) which carries out self-multiplication, with the pluripotency which can specialize in functional cells, such ıs a lymphocyte, an erythrocyte, and a platelet, and such pluripotency maintained, and it specializes and matures ıt various corpuscle cells while it performs self-renewal so that a pluripotency hematopoietic stem cell may not ɔe drained with a hematogenous controlling mechanism. From many old researches, the differentiation to each ;orpuscle sequence is oriented by differentiation decision of a multistage story, and the process in which a ɔluripotency hematopoietic stem cell specializes to various corpuscle cells is summarized like drawing 1 .
'0003] a pluripotency hematopoietic stem cell should be first oriented with two sequences of a myelocyte ;ystem and lymphocytic series, specialize to a bone marrow system stem cell (CFU-GEMM) and a lymphoid ;tem cell, respectively, and a bone marrow system stem cell should pass BFU-E and CFU-E further -- pass CFC-MEG to an erythrocyte -- pass EO-CFC in neutrophil leucocyte -- pass CFU-GM in eosinophile leucocyte -- become monocyte, neutrophil leucocyte, and basophilic leucocyte, and a lymphoid stem cell should pass a ɔre-T cell -- pass a pre-B cell in a T cell -- it becomes a B cell. About the specific approach of the various ɔrecursor cells to be derived a bone marrow system stem cell and from now on, the so-called colony formation assay which specifies these cells according to the description of the colony made in the half-fluidity culture nedium under existence of various cytokine is known. It is possible to specify the precursor cell of bone marrow ;ystems, such as granulocyte and a macrophage colony forming cell (CFU-GM) which is the granulocyte, the erythron, the monocyte system, megakaryocyte system colony forming cell (CFU-GEMM), and precursor cell which are a bone marrow system stem cell, an erythrocyte burst formation cell (BFU-E), a megakaryocyte colony forming cell (CFU-MEG), and an eosinophile leucocyte colony forming cell (EO-CFC), by this approach (Exp.Hematol., 11, 721, 1983). On the other hand, it is shown clearly that the so-called hematopoietic stem cells, such as a pluripotency hematopoietic stem cell, and a lymphoid stem cell, a bone marrow system stem cell, exist mainly in bone marrow, cord blood, etc., and it exists also in peripheral blood further (Blood, 87, 3082-3088, 1996). Although the attempt separated from a living body as a cell of homogeneity is performed in order that many researchers may clarify the stereo of these hematopoietic stem cells, in addition, current was not successful. It is said that pluripotency hematopoietic stem cells are conditions with indispensable having the "hematogenous reconstruction ability" which can build again all the corpuscle cell lineage trains that should be produced by the individual when the corpuscle cell which contains a hematopoietic stem cell by radiation

radiation is transplanted to the individual annihilated completely. (Blood, 75, 1941, 1990) since the precursor ell which forms a colony out of a living body is condensed in Homo sapiens by the cell population which has iscovered CD34 molecule which is a cell surface antigen, When Berenson and others tries transplantation of :D34 positivity cell to the cancer patient who did corpuscle cell extinction processing, reconstruction of the ematopoietic system is accepted. Also clinically, it came (Blood, 77, 1717, 1991) to be admitted that the luripotency hematopoietic stem cell which has hematogenous reconstruction ability is contained in the nsemble of CD34 positivity cell. However, for CD34 positivity cell, the attempt which carries out fractionation o a subset further since precursor cells other than a hematopoietic stem cell are also included is ******. Consequently, when most hematopoietic stem cells to a CD34 positivity CD38 negative cell population were ontained, they became Mr. idea **s (Blood, 77, 1218-1227, 1991, Proc.Natl.Acad.Sci.USA., 90, 8707-8711, 1993, Blood, 83, 1515-1526, 1994). moreover, these HPP-CFC since the high fecundity colony forming cell (HPP-CFC) orrelates with hematogenous reconstruction ability well as a result of analyzing the corpuscle of a mouse ccording to the above-mentioned colony formation assay -- a pluripotency hematopoietic stem cell -- very uch -- relation -- the deep thing became clear (Exp.Hematol., 10, 26-35, 1982). The cell with the same said of lomo sapiens was clarified, and it was recently shown that this is a precursor cell more immature than CFU-GEMM (Blood, 74, 609, 1989). When HPP-CFC was seen with the cell surface marker, it was condensed by CD34+++ cell fractionation, and the very immature thing was clarified (Blood, 81, 41-48, 1993). Moreover, since HPP-CFC is condensed by the CD34 positivity CD38 negative cell population, (Blood, 83, 3170-3181, 1994), and HPP-CFC can be called good index by which a hematopoietic stem cell is evaluated out of a living body.

0004] Mainly existing mostly in bone marrow was known, and by performing a bone marrow transplantation herapy, the so-called hematopoietic stem cells including the pluripotency hematopoietic stem cell which is a ell which has hematogenous reconstruction ability fixed to the bone marrow of a recipient (recipient) the ematopoietic stem cell which produces various corpuscle cells over a lifetime from the provider (donor), and vere considered that it can cure the various diseases relevant to blood completely. Although it was an xperimental therapy method in early stages, in current, it became the established cure (Jpn.J.Pediatr.Hematol., , 492, 1994). In current, the bone marrow transplantation therapy is performed to diseases, such as neoplasm ature hemopathies including acute leukemia, and serious illness immune disorder, adenosine deaminase leficiency, aplastic anemia. Furthermore, the transplantation using the peripheral blood which prescribes colony timulating factor pharmaceutical preparation (CSF) for the patient, and contains a hematopoietic stem cell is lso spreading as it becomes clear that these hematopoietic stem cells exist also in peripheral blood with small uantity (J.Hematother., 2, 513, 1993, Lancet, 341, 1482, 1993). Since a bone marrow transplantation needs a lot of bone marrow cells, to a thing with the large burden to a donor's mind and body, the burden to mind and body s mitigated to a donor, and this approach has the advantage that recovery of a leucocyte and a platelet is early. Moreover, it was shown clearly that cord blood recently contained a hematopoietic stem cell comparable as bone narrow, and it was shown clearly that it was useful for a transplantation therapy (New England J.Med., 335, 157, 1996). Compared with bone marrow or peripheral blood, the incidence rate of cord blood of serious illness acute graft versus host disease (GVHD) is low, and the usefulness is expected. However, in the case of cord blood, ittle of output is made into a problem and considered to be implantable by the cord blood originating in one ndividual by only the recipient to the weight of about 40kg (Blood, 87, 3082, 1996). In transplantation, the ecurrence by mixing of the onset of GVHD by mixing of the T cell of the donor origin and the cancer cell in the ase of autograft poses a problem (Lancet, 341, 85, 1993). on the other hand -- a hematopoietic stem cell -- CD -- 34 positivity, especially, it can condense as a cell population of 34 or so CD positivity cell, and CD34 positivity stem cell transplantation came (Blood, 77, 1717, 1991, J.Clin.Oncol., 12, 28, 1994) to be performed from possibility that an unnecessary cell will moreover be removable by the end of (Hematol.Oncol.Ann., 2, 78, 1994), and today. With such an advance, the certainty of a transplantation therapy is raised, and maintenance of the hematopoietic stem cell bank originating in many donors is becoming pressing need in order to perform more efficient operation (Transplant.Proc., 24, 3032-3034, 1992). The attempt which amplifies such a hematopoietic stem cell efficiently is performed to coincidence (Blood, 87, 3082-3088, 1996). Moreover, although the expectation for transplantation application is growing similarly about the cord blood containing many immature hematopoietic stem cells, since there is little output as above-mentioned, the system which amplifies a hematopoietic stem cell is expected (Blood, 87, 3082-3088, 1996). Thus, a hematopoietic stem cell can be said that stability and the construction of a system which can come to hand certainly are the problems to affect a life, and is the technical problem which should finish being socially important for the patient of said intractable hemopathy which does not have a cure in addition to transplantation.

0005] Since it is shown clearly that it is contained in CD34 positivity cell population, the various precursor cells lerived from hematopoietic stem cells including a pluripotency hematopoietic stem cell and these hematopoietic :tem cells are suitable to dissociate and condense, and are used as a start ingredient for magnification (Blood, :7, 3082-3088, 1996). 2% of these CD34 positivity cells exists from one in bone marrow or the blood cell of cord ›lood. It is a basic principle that separation of CD34 positivity cell collects the electropositive cells which have he antibody which recognizes CD34 molecule of cell surface, and reactivity. Carry out the indicator of the CD34 ıntibody with a biotin or a magnetic bead, and it is made to react with a cell population to separate. The ıpproach an avidin bead and a magnet recover CD34 positivity cell after that, respectively, After putting a cell ıto the culture instrument which carried out the coat of the CD34 antibody and removing CD34 antibody and he cell which does not react, The method of collecting CD34 positivity cells is often used, and even if it is vhich approach, CD34 positivity cell which does not have a difference qualitatively is recoverable (Hematother., :, 333, 1993, Exp.Hematol., 21, 585, 1993). In clinical, the device using CD34 antibody which carried out the nagnetic indicator, and a magnet is developed, and it is recognized as a rare disease therapy instrument in the ›hysic council even in Japan. Although magnification of CD34 positivity cell is generally performed in liquid :ulture, since the specific growth factor to each hematopoietic stem cell or precursor cell is not clear, the :ombination of the various cytokine which acts on each corpuscle cell is used, and many reports are made until ıow (Blood, 83, 1717, 1996). In magnification of CD34 positivity cell by the combination of cytokine Although the ıumber of whole blood spherocytes after culture increases, induction promotion of the differentiation is carried ›ut during culture. While the CD34 negative corpuscle cell which carried out the last differentiation as a result ncreases, it is known that most CD34 positivity cell itself [used as the source of magnification] will tend to be drained (Blood, 87, 3082-3088, 1996). In magnification of CD34 positivity cell reported until now, magnification of CFU-GM which is a precursor cell is made into the index in many cases. For example, Haylock and others :ultivated the peripheral blood CD34 positivity cell on the 14th under existence of interleukin 1 beta (IL-1beta) ınd IL-3, IL-6, a granulocyte-macrophage colony form origin child (GM-CSF), a granulocyte colony form origin :hild (G-CSF), and a stem cell factor (SCF), and it is reported that CFU-GM was amplified 20 to 60 times (Blood, 80, 1405, 1992). Moreover, there is also a report that CD34 positivity cell was cultivated for seven days ınder existence of IL-3, IL-6, SCF, G-CSF, and GM-CSF and CFU-GM was amplified 57 times (Blood, 82, 3600-3609, 1993). Moreover, the report of magnification of CFU-GEMM which is a bone marrow system stem cell was ılso carried out, and Brugger and others cultivated the peripheral blood CD34 positivity cell for 12 days by IL-1beta, IL-3, IL-6, interferon gamma (IFNgamma), and erythropoietin (EPO), and amplified CFU-GEMM 250 times (Blood, 81, 2579-2584, 1993). Moreover, magnification of CFU-GEMM also has a report of being possible, also by :ulture by SCF and IL-6, and fusibility IL-6 receptor (sIL-6R) (WO 96/No. 15230 official report). Since it was ıimed at the bone marrow system stem cell (CFU-GEMM) to which differentiation already came by the above ·eport although it was CFU-GM whose orientation in a specific cell lineage train is the already determined ›recursor cell, and a hematopoietic stem cell, it was what is hard to be called magnification of the hematopoietic stem cell which has hematogenous reconstruction ability. Therefore, although the analysis using the appraisal ·nethod which detects the cell (LTC-IC) which makes long term culture possible as a near cell identification method by the pluripotency hematopoietic stem cell rather than CFU-GEMM was tried Whether it amplifies very ›nly and only by being maintained Remarkable magnification was not accepted like CFU-GM (Blood, 81, 661, 1993, Blood, 75, 2118, 1990, Blood, 81, 2579, 1993, Blood, 84, 2898, 1994). Moreover, above-mentioned HPP-CFC is known as another appraisal method which detects a cell unriper than CFU-GEMM together with LTC-IC. In magnification of HPP-CFC HPP-CFC and LTC-IC are condensed for Srour and others by the a little less than 123 bone marrow CD34 positivity HLA-DR negative CD15 negative rhodamine positivity cell population -- finding out (it Cytometry(ing) Blood, 79, 634, and 1992 --) If 12, 179, 1991, and this cell are cultivated for four weeks by the fusion protein (PIXY321) of SCF, and IL-3 and GM-CSF, it will have reported that HPP-CFC amplifies 5.5 times (Blood, 81, 661-669, 1993). However, the magnification scale factor of the difficulty was low in the top where incubation period is long. Furthermore, Lu and others finds out that HPP-CFC is condensed by the CD34+++ cell contained at about 20% of a rate in CD34 positivity cell population (Blood, 81, 41-48, 1993). this cell -- IL-1 -- when cultivated under alpha, SCF, and IL-3 existence, it reported that HPP-CFC could amplify 160 times in seven days (Blood Cells, 20, 455-467, 1994). It is thought that it sees rather than other amplifying methods, and one 5 times the upper magnification scale factor of this is highly estimated by this amplifying method since a start ingredient is in the phase to which concentration went nearby [about 5-time] from the usual CD34 positivity cell. Moreover, there is a difficulty that HPP-CFC falls rapidly to 1/80 on the 14th day of culture, and handling is not easy, either. Therefore, it is not necessary to condense CD34 positivity cell and, it is

tabilized simple, and the approach of amplifying is considered to be useful by the use on clinical [, such as ransplantation,].

0006] On the other hand, as the magnification approach of CD34 positivity cell, the stromata cell of the Homo apiens bone marrow origin is stock-ized, and maintenance and the approach of proliferating of a hematopoietic tem cell and a precursor cell are tried on this (Exp.Hematol., 22,482-487 (1994)). However, in order to hardly mplify or to decrease from the time of the beginning rather, even if it transplants the stem cell immature in rder that CD34 positivity cell may specialize by this approach itself, it has a possibility that a ****** cell may e drained soon, and is incongruent as a technique for transplantation. Moreover, as an exception method, even there is no contact into a stromata cell, there is also a report that it can amplify by cultivating only by the umoral factor produced from stromata (the [international patent application public presentation] No. 93/20184 fficial report). However, in order not to carry out self-multiplication of the hematopoietic stem cell only by pecializing in this approach, as a technique for transplantation by the same reason as the above-mentioned pproach, it is incongruent. Moreover, JP,7-504331,A has the publication of the purport growth of CD34 ositivity cell under coexistence with several sorts of other cytokine is accepted to be, using a certain kind of an nterstitial cell and the leukemia inhibitor (LIF) as indispensable cytokine. However, incubation period is several veeks and a long period of time, and it is thought that it is difficult for using it by clinical and going. Moreover, he example evaluated by the absolute number of a cell is also reported about magnification of CD34 positivity ell. For example, if Sato and others cultivates a peripheral blood CD34 positivity cell under existence of IL-3, L-6, SCF, G-CSF, and GM-CSF, the CD34 positivity CD33 positivity cell will have reported that magnification vas possible about 300 times (Blood, 82, 3600-3609, 1993). If the reinforcement (amount) of a manifestation of D34 molecule in this report is seen, it is a little less than 34 CD positivity, and since the field which laps with a D34 negative cell population is also included, it will be hard to call it evaluation of the absolute number of CD34 ositivity cell purely. moreover, the thing for which Srour and others will be amplified 2.9 times as a CD34 ositivity cell population if a a little less than 123 bone marrow CD34 positivity HLA-DR negative CD15 negative hodamine positivity cell population is cultivated for seven days by SCF and PIXY321 -- having reported (Blood, 1, 661-669, 1993) -- as a magnification scale factor, it was low. As mentioned above, it can be said that it is lesirable in the cell population of CD34 positivity to make 34 or so CD positivity cell into an index in the ematogenous reconstruction ability evaluation in an absolute number at least if immature stem cells including PP-CFC are seen from it being 34 or so CD positivity cell.

0007]

Problem(s) to be Solved by the Invention] As stated above, magnification of CD34 positivity cells including the luripotency hematopoietic stem cell which has hematogenous reconstruction ability has been a technical roblem with how big the differentiation started in the process which amplifies is controlled and growth of the D34 positivity cell itself is enabled [how]. Moreover, it waited for development of the cultivation which can be tabilized and amplified by the shortest possible incubation period simple out of a living body. It is reported that he stromata cell strain used by this invention is a cell originating in mouse bone marrow and a spleen, and it is he outstanding stromata cell strain which the cell strain named especially HESS-5 also in these cell strains guides growth of a mouse bone marrow precursor cell, and supports the long term culture of a bone marrow system and a B lymphocyte system by the culture condition (Leukemia, 10,803-812, 1996). In the invention in this application, this cell strain and other cell strains are applied to magnification of a Homo sapiens CD34 positivity cell. About the effectiveness what kind of operation to exert on CD34 positivity cell in which HESS-5 cell strain and other mouse stromata cell strains included the human hematopoietic stem cell, there was no knowledge until now and it became clear for the first time in the invention in this application.

[0008]

[Means for Solving the Problem] this invention persons put under coexistence with a stromata cell the hematopoietic stem cell which is CD34 positivity cell, as a result of inquiring wholeheartedly, in order to solve the above-mentioned technical problem. If it puts in another way and a stromata cell of a certain kind exists, a hematopoietic stem cell can be proliferated notably and cytokine will be mixed in a nutrition culture medium if needed It came to complete a header and this invention for proliferating further the hematopoietic stem cell containing the hematopoietic stem cell and/or HPP-CFC which are CD34 positivity cell. That is, according to this invention, a hematopoietic stem cell can be increased under coexistence of a certain kind of stromata cell. If it puts in another way, self-renewal of the hematopoietic stem cell can be further carried out by making a certain kind of stromata cell exist by being able to proliferate a hematopoietic stem cell and mixing one sort or two sorts or more of cytokine in a nutrition culture medium if needed. Moreover, growth is similarly guided about

he bone marrow system precursor cell group to which the differentiation phase progressed a little from the ıematopoietic stem cell. Furthermore, according to this invention, using the culture instrument which embodied he principle of cultivation, only a required number can carry out self-renewal of the hematopoietic stem cell, ınd the cell increased very easily can be extracted. In addition, although not necessarily limited to these cells ıspecially as a CD34 positivity cell, especially 34 or so CD positivity cell may be the organization which contains ıot only a pure blood cell but such a cell preferably.

0009] That is, the invention in this application is invention as following.

1) The manufacture approach of CD34 positivity cell of the Homo sapiens characterized by proliferating this ıomo sapiens's CD34 positivity cell by cultivating in the nutrition culture medium containing the stromata cell of he mammalian origin which has the capacity to proliferate human CD34 positivity cell for human CD34 positivity ;ell.

2) The manufacture approach given in the above (1) whose CD34 positivity cell manufactured by culture is ;haracterized by being 34 or so CD positivity cell.

3) The manufacture approach given in the above (1) or the above (2) characterized by CD34 positivity cell being CD34 positivity cell originating in cord blood, bone marrow, liver, a spleen, or peripheral blood.

(4) The manufacture approach given in either the above (1) characterized by being the organization of a mouse which a stromata cell includes for the stromata cell or this stromata cell of the mouse origin thru/or the above (3).

A stromata cell (5) HESS-1, HESS-5 (international deposition number: FERM BP-5768), HESS-18 (international ıeposition number: FERM BP-6187), HESS-M28 (international deposition number: FERM BP-6186), That they ıre one sort or two sorts or more of stromata cells chosen from the group which consists of a stromata cell of :he mouse origin respectively named SSXL CL.1, SSXL CL3, SSXL CL.7, SSXL CL.9, and SSXL CL.17 The manufacture approach given in the above (4) by which it is characterized.

(6) The manufacture approach given in the above (4) characterized by being the stromata cell chosen from the group which a stromata cell becomes from the stromata cell strain of the mouse origin respectively named HESS-5 (international deposition number: FERM BP-5768), HESS-18 (international deposition number: FERM BP-6187), HESS-M28 (international deposition number: FERM BP-6186), and SSXL CL.3.

(7) The manufacture approach given in either the above (1) characterized by cultivating under existence of cytokine thru/or the above (6).

(8) The manufacture approach given in the above (7) characterized by cytokine being one sort or two sorts or more of cytokine chosen from the group which consists of interleukin 3, stem cell factor (SCF), granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), flk2/flt3-ligand, and macrophage origin inflammatory protein 1alpha (MIP-1alpha) and erythropoietin (EPO).

(9) The manufacture approach given in either the above (1) with which CD34 positivity cell is characterized by being cultivated in the state of a stromata cell, a contact condition, a non-contact condition, or indirect contact thru/or the above (8).

(10) The manufacture approach given in either the above (1) with which CD34 positivity cell is characterized by being cultivated in the state of a stromata cell and indirect contact thru/or the above (8).

(11) Human CD34 positivity cell obtained by either the above (1) thru/or the above (10) by the manufacture approach of a publication.

(12) The physic constituent which consists of a CD34 positivity cell of the Homo sapiens obtained by either the above (1) thru/or the above (10) by the manufacture approach of a publication, and support which may be permitted by the pharmaceutical-sciences target.

(13) The stromata cell strain of the mouse origin identified by international deposition number FERM BP-6187.

(14) The stromata cell strain of the mouse origin identified by international deposition number FERM BP-6186.

(15) It is a culture instrument for carrying out growth culture of the CD34 positivity cell which is the culture instrument which consists of at least one base material for supporting the cell held in the culture container and this culture container, and is characterized by for this base material to consist of support for making the supporting lamella and this supporting lamella for carrying out seeding support of either [at least] CD34 positivity cell or a stromata cell into a culture medium fix to a container.

(16) The 1st film which it is [film] an instrument for cultivating a cell, and the instrument:(a) nutrition culture medium and cytokine which are characterized by consisting of following members at least can be penetrated [film], and cannot pass a cell;

(b) the 2nd film (here -- this -- two film) which it can be arranged [film] at the top-face side of this 1st film,

an make a carbon dioxide able to penetrate, and cannot make a liquid penetrate the following -- opening by ubing of (d) being arranged -- removing -- this -- it is arranged so that the electric shielding system which has ontent volume which a liquid does not leak besides this instrument may be formed between two film. ;
c) the 3rd film (here -- this -- two film) which it can be arranged [film] at the inferior-surface-of-tongue side f this 1st film, can make a carbon dioxide able to penetrate, and cannot make a liquid penetrate the following -- pening by tubing of (e) being arranged -- removing -- this -- it is arranged so that the electric shielding ystem which has content volume which a liquid does not leak besides this instrument may be formed between wo film. ;
d) Tubing of the 2nd ** which it is arranged [**] between this 1st film and the 2nd film, is arranged [**] etween 1st tubing; which can make a liquid, a nutrition culture medium, and a cell pour in or discharge and the e) this 1st film, and the 3rd film, and can make a liquid, a nutrition culture medium, and a cell pour in or lischarge.
17) either of the electric shielding systems which have the content volume formed between the 1st film and the 2nd film or between the 1st film and the 3rd film -- this -- an instrument given in the above (16) characterized by being formed by arranging one side of two film at one side of an insoluble frame, and arranging the film of another side in other fields of this frame.
18) An instrument given in the above (16) or the above (17) characterized by including the nutrition culture medium in each of the electric shielding system which has the content volume formed between the 1st film and the 2nd film and between the 1st film and the 3rd film, and the cell having pasted one field of the 1st film.
19) An instrument given in the above (18) whose cell is characterized by being the stromata cell of mammalian.
20) An instrument given in the above (16) or the above (17) characterized by containing the stromata cell of mammalian in either of the electric shielding systems which have the content volume formed between the 1st film and the 2nd film or between the 1st film and the 3rd film.
21) An instrument given in the above (19) or the above (20) characterized by a stromata cell being a stromata cell which has the capacity to proliferate human CD34 positivity cell.
22) An instrument given in the above (21) characterized by being the stromata cell chosen from the group which a stromata cell becomes from the stromata cell strain of the mouse origin respectively named HESS-5 (international deposition number: FERM BP-5768), HESS-18 (international deposition number: FERM BP-6187), HESS-M28 (international deposition number: FERM BP-6186), and SSXL CL.3.
23) The manufacture approach of CD34 positivity cell of the Homo sapiens characterized by using the instrument of a publication for either the above (15) thru/or the above (22).
[0010]
[Embodiment of the Invention] Here, the "hematopoietic stem cell" used for this invention is a cell which has hematogenous reconstruction ability while having the capacity which specializes in all kinds of corpuscle, and it mainly exists in bone marrow, cord blood, a spleen, or liver, and exists also in peripheral blood with a minute amount. These hematopoietic stem cells are 34 or so CD positivity cells, and a high proliferation potential colony forming cell (High-Proliferative Potential Colony-Forming Cells (HPP-CFC)) is also included by this in this invention. A "stem cell" means the lymphoid stem cell and bone marrow system stem cell (CFU-GEMM) which specialized a pluripotency hematopoietic stem cell and from now on. These cells are CD34 positivity cells.
[0011] Although the blood cell of each network cannot identify a "precursor cell" on a differentiation morphology target from a pluripotency hematopoietic stem cell, it means the cell which cannot already specialize only in the blood cell of one directions, such as erythron. Specifically, they are a platelet colony forming cell (CFC-MEG), an eosinophile leucocyte colony forming cell (EO-CFC), a granulocyte monocyte colony forming cell (CFU-GM), an erythropoiesis cell (BFU-E, CFU-E), a pre-T cell, a pre-B cell, etc. Each of these is CD34 positivity cells.
[0012] A "functional cell" means the cell which has a function as a corpuscle cell. Specifically, they are an erythrocyte, a platelet, eosinophile leucocyte, monocyte, neutrophil leucocyte, eosinophile leucocyte, a T cell, a B cell, etc.
[0013] A "differentiation anti-original table solid pattern" means the phenotype of the differentiation antigen which exists on a Homo sapiens corpuscle cell preferably on the cell surface of mammalian. Usually, this kind of antigen is classified with the number of CD. By the abbreviation for cluster of differentiation, CD means the 1 lump (cluster) of the antigen recognized by the monoclonal antibody. Specifically, CD34, CD4, CD8, CD10, CD13, CD19, CD33, CD38, etc. can be mentioned. Thy-1, HLA-DR, etc. can be mentioned to others.
[0014] "Cell lineage" points out all the cultured cells after primary culture, and means a series of networks of the cell or cell population which existed in primary culture. This cultured cell may exist, where you could coexist

ith primary cell culture, and you could exist in the condition of having come into contact with mutually or
ıedia, such as liquids, such as water and an electrolyte, a culture medium, and culture medium, are minded.
0015] An "organization" specializes in the specific direction and means a cell population with the same function
nd a gestalt. As an organization containing a stromata cell, bone marrow, a spleen, etc. can specifically be
ıentioned.
0016] "CD34 positivity cell" means the cell which has discovered CD34 which is one of the antigen phenotypes,
nd precursor cells, such as stem cells, such as hematopoietic stem cells, such as a pluripotency hematopoietic
tem cell and HPP-CFC, a lymphoid stem cell, and a bone marrow system stem cell, a pre-T cell, a pre-B cell,
FU-E, CFU-E, CFU-MEG, EO-CFC, and CFU-GM, specifically correspond to this.
0017] "34 or so CD positivity cell" means the cell which has discovered especially strongly CD34 which is one
f the antigen phenotypes, and means CD34 positivity cell population which specifically contains more high
roliferation potential colony forming cells (High-Proliferative Potential Colony-Forming Cells), pluripotency
ematopoietic stem cell itself, or these cells.
0018] "CD34 shade sexual cell" means the functional cell which has not discovered CD34 which is one of the
ntigen phenotypes. The cell of the T cell sequence after the pre-T cell which specifically contains a T cell, the
ell of the B cell sequence after the pre-B cell containing a B cell, The cell of the erythrocyte sequence after
FU-E containing an erythrocyte, the cell of the platelet sequence after CFC-MEG containing a platelet, It is
he cell of the monocyte after CFU-GM containing the cell or the monocyte, neutrophil leucocyte, or basophilic
eucocyte of an eosinophile leucocyte sequence after EO-CFC containing eosinophile leucocyte, neutrophil
eucocyte, or a basophilic leucocyte sequence. Depending on the case, it only writes respectively "+" (plus),
high+", "low", and "-" (minus). [the vocabulary which is used in this application and "negative" negative / the
bove "positivities", a "a little more than positivity", a "weak positivity", and / becoming] or [for example, /
aving discovered CD34 strongly (strong positivity), and having discovered CD38 weakly with
CD34high+CD38low/-",] -- or what (a weak positivity or negative) is not discovered is meant.
0019] A "stromata cell" points out the substrate cell or interstitial cell originating in bone marrow, a spleen,
tc., and in the invention in this application, if it is the stromata cell which has the capacity to increase human
D34 positivity cell, any stromata cells can be used. For example, the stromata cell of the mouse origin
espectively named HESS-1, HESS-5 (international deposition number: FERM BP-5768), HESS-18 (international
leposition number: FERM BP-6187), HESS-M28 (international deposition number: FERM BP-6186), SSXL CL.1,
SXL CL3, SSXL CL.7, SSXL CL.9, and SSXL CL.17 is illustrated. Preferably, it is HESS-5 (international
leposition number: FERM BP-5768), HESS-18 (international deposition number: FERM BP-6187), HESS-M28
international deposition number: FERM BP-6186), or SSXL CL3, and they are HESS-5 (international deposition
umber: FERM BP-5768), HESS-18 (international deposition number: FERM BP-6187), and HESS-M28
international deposition number: FERM BP-6186) especially preferably.
0020] A "culture cell strain" is a cell originating in a living body's organization, an organ, etc., and is the cell
which became possible [carrying out subculture out of a living body] by gaining infinity autonomous replication
ability by cultivating out of a living body. Generally the stock originating in a single cell is made, and this is called
a cell strain. The fibroblast stock, the epithelial cell stock, etc. are known by the culture cell strain.
0021] A desired cell and the 2nd desired cell separate distance in a culture medium, and exist separately, and a
"non-contact condition" shows the condition of having not come into contact with directly mutually.
0022] A "contact condition" may specifically be in a desired cell, the 2nd cell, and the condition that CD34
positivity cell and stromata cells, such as a hematopoietic stem cell, mix and exist in arbitration, and may be in
which condition in the condition which a desired cell and the 2nd desired cell have suspended in a culture
medium (culture medium) in this case, the condition tidily located in a line in the shape of a layer, and the
condition that the cell of another side was hidden between one cells.
[0023] As for an "indirect contact condition", a desired cell, the 2nd cell, and the condition that CD34 positivity
cell and a stromata cell separate, respectively, and specifically exist in a front-face and rear-face side in the
shape of a layer through a microporous supporting lamella are said.
[0024] Although a natural medium, a semisynthetic medium, a synthetic medium, a solid medium, a semisolid
medium, a liquid medium, etc. are mentioned, as long as it seems that a "nutrition culture medium" is used in
order [including self] to make it increase, specialize, mature or save, and CD34 positivity cell defined as the
above-mentioned is usually used for a cell culture, it may be what kind of culture medium. If an example is given,
an alpha-MEM culture medium, RPMI-1640 culture medium, or an MEM basal medium can mention, for example.
According to sodium, a potassium, calcium, magnesium, Lynn, chlorine, amino acid, a vitamin, hormone, an

ntibiotic, a fatty acid, sugar, or the purpose, other chemical entities or a biogenic substance like a blood serum an also be contained as a fundamental component.

0025] A "base material" is for supporting CD34 positivity cell and/or a stromata cell in a culture container, and : consists of the "supporting lamellas" and the "support" like the following. It is called the cel culture insertion which carried out the silk hat configuration preferably especially.

0026] "Supporting lamellas" is a desired cell, the 2nd cell, and a member used in order to specifically separate D34 positivity cell and a stromata cell. As a supporting lamella, a microporous thing is desirable, and, as for the nagnitude of the hole at this time, it is desirable that they are a desired cell, the 2nd cell, and the hole of nagnitude that both stromata cell and CD34 positivity cell can specifically pass. You may be the shape of a film nd the porous film which can pass electrolytes, such as water, sodium ion, and a chlorine ion, etc., may be film ike the cellophane which cannot pass protein, hormone, etc., and can specifically pass electrolytes, such as vater, sodium ion, and a chlorine ion, protein, hormone, etc. The porous film of macromolecules, such as protein, such as electrolytes, such as water, sodium ion, and a chlorine ion, and cytokine, and hormone, which can be passed and a part of both cell or one cell can project in the shape of a projection is preferably desirable.

0027] Moreover, maintenance, survival, differentiation and maturation, although self-renewal is carried out, as ong as a stromata cell can maintain and survive and CD34 positivity cell does not check a membranous material t all, it may be what kind of material. As a material, polyethylene terephthalate, a polycarbonate, etc. are specifically mentioned. Furthermore, the film may be the configuration of the arbitration which changes without a configuration's being fixed, and a configuration's may be fixed. The configuration of the arbitration which changes vithout the configuration and globular form by which a part like hemispherical besides the shape of a plane and a vave and box-like is opened wide, the shape of a tube, and a configuration being specifically fixed is sufficient. At this time, in the case of the film of closed systems (sealing system), such as a globular form, a desired cell is put into the interior of membranous, and it puts the 2nd cell into a membranous outside, or puts the 2nd cell into he interior of membranous conversely, and should just put a desired cell into a membranous outside. Moreover, nembranous hardness may be what kind of thing. Moreover, if the minute perforated board which comes to punch the shape of the shape of reticulated and textile fabrics and a nonwoven fabric, the shape of paper, and nicroporosity is mentioned as an example of a microporous base material, it can **.

0028] "Support" is a member for fixing these "a supporting lamella" to a culture container, and various things can be used as occasion demands. The susceptor for laying on it the piece of support for fixing to ledged [which s fixable to the suspender for specifically hanging a base material in a culture container and a vessel wall], or a supporting lamella etc. can be mentioned. . (see drawing 2 thru/or drawing 4 in detail).

0029] "Coexistence" coexistence [which is used by the invention in this application] The becoming vocabulary says a desired cell, the 2nd cell, and the condition that CD34 positivity cell and the stromata cell exist in the state of arbitration in one culture medium (culture medium), and specifically includes the above contact conditions, a non-contact condition, and an indirect contact condition. However, in the below-mentioned example, especially, as long as there is no notice, a contact condition is meant.

[0030] "Immobilization or adhesion" shows the condition that a certain matter always contacts other matter, becomes settled in one place, and does not move mutually. You may specifically be in the condition which the condition that it is always in contact with the supporting lamella which a certain cell (they are CD34 positivity cell or a stromata cell as a concrete cell) by which seeding was carried out showed above is said, and this cell always contacts this supporting lamella, and does not become settled and move to one place, and this cell may be in the condition which is moving while always contacting this film.

[0031] "Cytokine" is the protein sex factor which is emitted from a cell and carries biotaxis. It is the matter in which the control action of an immune response, antitumor action, an antiviral action, the accommodation of cell proliferation and differentiation, etc. are shown. Specifically Interleukin 1 (IL-1), interleukin-2 (IL-2), Interleukin 3 (IL-3), interleukin -4 (IL-4), Interleukin -5 (IL-5), interleukin -6 (IL-6), Interleukin -7 (IL-7), interleukin -8 (IL-8), Interleukin -9 (IL-9), interleukin -10 (IL-10), Interleukin -11 (IL-11), interleukin -12 (IL-12), Interleukin -13 (IL-13), interleukin -14 (IL-14), Interleukin -15 (IL-15), interleukin -16 (IL-16), Interferon-alpha (IFN-alpha), interferon beta (IFN-beta), Interferon gamma (IFN-gamma), a granulocyte colony-stimulating factor (G-CSF), A granulocyte-monocyte colony stimulating factor (GM-CSF), a monocyte colony stimulating factor, A granulocyte-macrophage colony-stimulating factor, an eosinophile leucocyte colony stimulating factor, A platelet colony stimulating factor, a stem cell factor (SCF), a stem cell growth factor, flk2/flt3-ligand, a leukemia inhibition (inhibition) factor, erythropoietin (EPO), Macrophage origin inflammatory protein 1alpha (MIP-1alpha) etc. is mentioned. Interleukin 3, stem cell factor (SCF), granulocyte colony-stimulating factor, granulocyte-

nacrophage colony-stimulating factor, flk2/flt3-ligand, and MIP-1alpha or erythropoietin is mentioned preferably.

0032] It is called a "cryopreservation agent" also with a frost damage inhibitor and a frost damage defensive ubstance. The matter added into a culture medium (culture medium) in order to mitigate the frost damage in the ase of a living thing cell and specifically saving a stromata cell or CD34 positivity cell, living in the state of reezing is meant. Specifically, they are a glycerol, ethylene glycol, dimethyl sulfoxide (DMSO), cane sugar, a lucose, a polyvinyl pyrrolidone (PVP), etc.
0033] "Culture containers" is a desired cell and a container used when specifically proliferating CD34 positivity ells, such as a hematopoietic stem cell, a stromata cell can maintain and survive, and maintenance, survival, lifferentiation and maturation, although self-renewal is carried out, as long as CD34 positivity cell does not revent at all, the thing of what kind of material and a configuration may be used. As a material of a culture ontainer, specifically Glass, synthetic resin, natural resin, a metal, Plastics etc. is mentioned. As a configuration, pecifically The triangle pole, a cube, Multiple spindles, such as multiple columns, such as a rectangular arallelepiped, a triangular pyramid, and a square drill, the configuration of arbitration like a gourd, a globular orm, a semi-sphere, a cylinder (a base contains circular, an ellipse form, or a hemicycle), etc. can be mentioned, nd a configuration may be changed from a semi-sphere if needed during culture like a globular form. Culture nay be under an open condition and may be under a closing (sealing) condition.
0034] The visible conglomerate which left the "colony" from one cell by the solid medium, and was made is aid.
0035] "Differentiation" means the surface antigen molecule expressed by CD here changing, and becoming the ell of the next phase. It says that the front pattern of a differentiation antigen specifically changes from a nultipotential stem cell (CD34+CD38-) like a lymphoid stem cell (CD34+CD38+) or a bone marrow system stem ell (CD34+CD38+CD33+). From the pre-T cell or pre-B cell, and bone marrow system stem cell from a lymphoid tem cell to BFU-C From BFU-E to CFC-MEG, EO-CFC or CFU-GM, and CFU-E It says becoming monocyte, eutrophil leucocyte, or basophilic leucocyte from the platelet from the T cell from a pre-T cell, the B cell from a pre-B cell, the erythrocyte from CFU-E, and CFC-MEG, the good oxidation ball from EO-CFC, and CFU-GM (see drawing 1 about CD).
0036] Any film is usable if it is the film which has the property which proteins, such as a nutrition culture nedium for cultivating and maintaining a cell at least as the "1st film" in above-mentioned invention of (16) which is one of this invention, and cytokine, can be penetrated [property], and cannot pass a cell. The film which has many fine holes is specifically desirable, and the magnitude of a hole is the film which cannot be made to pass the cell to cultivate but penetrates electrolytes, such as liquids, such as water and culture medium, sodium ion, and a chlorine ion, and the above cytokine (it responds to a request and they are a lipid, sugar, etc.) by one side. Furthermore, as another mode, electrolytes, such as liquids, such as water and culture medium, sodium ion, and a chlorine ion, and the above cytokine (it responds to a request and they are a lipid, sugar, etc.) can be penetrated, and the film which a part of cell can project in the shape of a projection through this fine hole is mentioned. As a concrete example, 0.1-0.6 micrometers (micrometer) of 0.4-0.5-micrometer fine holes are mentioned preferably. as long as membranous reinforcement is maintained as the number of the fine holes of this film -- more possible ones -- desirable -- for example, 5-20 hole / cm -- /cm can be mentioned about 10 holes preferably. Moreover, as long as a cell can maintain and survive and does not check maintenance of a cell, survival, differentiation, maturation, and/or a duplicate as a membranous material, you may be what kind of material. Polyethylene terephthalate, a polycarbonate, etc. specifically mention and it is ****. Furthermore, the film may be the configuration of the arbitration which changes without a configuration's being fixed, and a configuration's may be fixed. Any film is usable if it is the film which has the property which a carbon dioxide can be penetrated [property] at least and cannot make a liquid penetrate as the "2nd film" in above-mentioned invention of (16) which is one of this invention, and the "3rd film." That is, a carbon dioxide required in order to cultivate a cell in the electric shielding system which has the content volume formed between said 1st film and 2nd film and/or between this 1st film and the 3rd film can be penetrated, and liquids, such as nutrition culture medium added in this electric shielding system, point out the film which it can avoid leaking (outside an instrument) out of this electric shielding system. In the electric shielding system which has the content volume formed between said 1st film and 2nd film and/or between this 1st film and the 3rd film, "tubing" in above-mentioned invention of (16) which is one of this invention is arranged in order to pour in a liquid and cells, such as culture medium, and to make a liquid and cells, such as culture medium, discharge from this electric shielding system. As this tubing, into this electric shielding system, if it seems that a liquid and cells, such as culture

nedium, can be poured in, and a liquid and cells, such as culture medium, can be made to discharge from this lectric shielding system, anythings can be used, for example, a silicon tube can be mentioned. Even if this ** annot be found, each of a two-layer electric shielding system is filled up with a nutrition culture medium, but ince the condition (condition that it is completely filled with the nutrition culture medium, and a gaseous phase loes not exist) of not including air in which system is desirable, the air included in each electric shielding system an be discharged through this tubing. The instrument which has a configuration which is illustrated by drawing 3 is contained in the instrument of ** (16) invention of this application. The cell culture in this application can e carried out as follows according to a conventional method. The need is accepted into a culture container. Sodium, a potassium, calcium, magnesium, The alpha-MEM culture medium which contained other chemical ntities or a biogenic substance like a blood serum according to Lynn, chlorine, amino acid, a vitamin, hormone, n antibiotic, a fatty acid, sugar, or the purpose, Under existence of the inside of nutrition culture media, such as RPMI-1640 culture medium or an MEM basal medium, and a stromata cell, the need -- responding -- 5 ng(s)/ml hru/or 200 ng(s)/ml -- desirable -- the interleukin 1 (IL-1) of concentration (10 ng(s)/ml thru/or 100 ng(s)/ml) -- Interleukin-2 (IL-2), interleukin 3 (IL-3), Interleukin -4 (IL-4), interleukin -5 (IL-5), Interleukin -6 (IL-6), nterleukin -7 (IL-7), Interleukin -8 (IL-8), interleukin -9 (IL-9), Interleukin -10 (IL-10), interleukin -11 (IL-11), nterleukin -12 (IL-12), interleukin -13 (IL-13), Interleukin -14 (IL-14), interleukin -15 (IL-15), Interleukin -16 IL-16), interferon-alpha (IFN-alpha), Interferon beta (IFN-beta), interferon gamma (IFN-gamma), A granulocyte colony-stimulating factor (G-CSF), a granulocyte-monocyte colony stimulating factor (GM-CSF), A monocyte colony stimulating factor, a granulocyte-macrophage colony-stimulating factor, An eosinophile leucocyte colony stimulating factor, a platelet colony stimulating factor, a stem cell factor (SCF), A stem cell growth factor, flk2/flt3-ligand, a leukemia inhibition (inhibition) factor, Cytokine, such as erythropoietin (EPO) and macrophage origin inflammatory protein 1alpha (MIP-1alpha) Under existence, Seeding of the CD34 positivity cell can be carried out, and 40 degrees C of CD34 positivity cells can be proliferated 30 degrees C thru/or by cultivating for ten days thru/or for 25 days preferably for five days thru/or for 30 days at 37 degrees C.

[0037] As concrete instantiation of arrangement of the stromata cell under culture, and CD34 positivity cell, contact cultivation, non-contact cultivation, and indirect contact cultivation are mentioned, and the culture approach is indicated concretely below. It is the approach of contacting CD34 positivity cells, such as a stromata cell and a hematopoietic stem cell, directly, and cultivating them in culture medium like the nstantiation of drawing 2 of this application drawing to the 1st. During culture, a stromata cell and CD34 positivity cell can contact vertical and horizontal arbitration directly in the shape of a layer mutually, and CD34 positivity cell can be hidden between a stromata cell and a stromata cell, or they can also carry out it. Conversely, a stromata cell can be hidden between CD34 positivity cell and CD34 positivity cell, or can also be carried out.

[0038] As illustrated to the 2nd at drawing 3 (a) of this application drawing thru/or (d), it is the culture approach in the condition, i.e., a non-contact condition, that separate with the supporting lamella which fixed the stromata cell and CD34 positivity cell to the container with various support, and the mutual cell does not touch. The drawing 3 (a) base material is the thing of the silk hat configuration by which a supporting lamella and support were formed in one. The thing of drawing 3 (b) increases a supporting lamella further, and is taken as two-layer. Then, the lower film is installed so that culture medium (culture medium) may be bisected in a culture container, the upper film may support it with support, and it may be installed as the culture medium (culture medium) in the condition, i.e., a culture container, that both the upper film and the lower film divide culture medium (culture medium) is trichotomized by the film of two sheets. In this case, there may be membranous support or there may be. [no] Moreover, maintenance, survival, differentiation and maturation, although self-renewal is carried out, as long as a stromata cell can maintain and survive and CD34 positivity cell does not check the material of support at all, it may be what kind of material. As a material, glass, synthetic resin, natural resin, or a metal specifically mentions, and it is ****. Moreover, drawing 2 (c) shows the case where a supporting lamella is hung to ledged in a container, and drawing 2 (d) shows the case where it supports from the bottom with the support which prepared the supporting lamella in the container base. Drawing 3 (a) is separated by the film of one sheet, and drawing 3 (b) is separated by the film of two sheets. Moreover, the support plate of the film on drawing 3 (b) and the support plate of the lower film may be the same support plates, and may be a respectively different support plate. Furthermore, you may be in the condition which the stromata cell and CD34 positivity cell replaced in drawing 3 (a) and (b). Both drawing 3 (a) and (b) of film may be only flat surfaces. You may be which case although what used the membranous support plate section as the film, and used the flat surface as matter which is not electrolyte ****, such as water, sodium ion, and a chlorine ion, a flat surface, and the membranous

:upport plate section were used as the film. Furthermore, the film attaches a support plate if needed, unless a ;lobular form, the shape of a tube, and a mutual cell contact, it may have and carry out the configuration of ırbitration, it can put CD34 positivity cell into the inside side of the film, and can also put a stromata cell into a nembranous outside. Conversely, a stromata cell can be put into the inside side of the film, and CD34 positivity :ell can also be put in outside.

0039] It is the approach of 3rd cultivating a desired cell (specifically CD34 positivity cell) and the 2nd desired :ell (specifically stromata cell) in the state of indirect contact through a supporting lamella like instantiation of drawing 4 (a), (b), and (c). Although a supporting lamella is supported with support, and a mutual cell passes the ilm and is not mixed, it is the approach of cultivating in the condition that both separate the film of each other ınd are located most in near. The thing of a silk hat configuration is preferably used like the case of drawing 3 by .he base material in drawing 4 (a). Drawing 4 (a) is separated by the film of one sheet, and drawing 4 (b) is ;eparated by the film of two sheets. Drawing 4 (b) is in the condition which both the cells of the 2nd cell specifically stromata cell) and a desired cell (specifically CD34 positivity cell) pasted up on one film, is in the :ondition that a mutual cell can be contacted and only the 2nd cell (specifically stromata cell) has pasted up on :he film of another side. A desired cell (specifically CD34 positivity cell) is pinched by the film of two sheets, and drawing 4 (c) is placed so that both film may be contacted. The 2nd cell (specifically stromata cell) is contacted :o the opposite field of the film of two sheets with which the desired cell (specifically CD34 positivity cell) :ouches. The condition of drawing 4 (c) is similar to the condition that CD34 positivity cell was hidden in the ;tromata cell. You may be in the condition which a desired cell (specifically CD34 positivity cell) and the 2nd desired cell (specifically stromata cell) replaced in drawing 4 (a), (b), and (c). Both drawing 4 (a) (b) and (c) of film nay be only flat surfaces. You may be which case although what used the membranous support plate section as :he film, and used the flat surface as matter which is not electrolyte ****, such as water, sodium ion, and a chlorine ion, a flat surface, and the membranous support plate section were used as the film. Furthermore, ınless it attaches a support plate if needed and a globular form, the shape of a tube, and a mutual cell contact, :he film has the configuration of arbitration, can put a desired cell (specifically CD34 positivity cell) into the nside side of the film, and can also put the 2nd cell (specifically stromata cell) into a membranous outside. Conversely, the 2nd cell (specifically stromata cell) can be put into the inside side of the film, and a desired cell (specifically CD34 positivity cell) can also be put in outside.

0040] the film with which the supporting lamella used by the 2nd and 3rd culture approaches has 1 or 2 thru/or a countless fine hole -- desirable -- the magnitude of the hole at this time -- a desired cell, the 2nd cell, and the hole of magnitude that both a stromata cell and CD34 positivity cell can specifically pass -- if it kicks, it will not become. You may be the film which can pass electrolytes, such as water, sodium ion, and a chlorine ion, etc., may be film like the cellophane which cannot pass macromolecules, such as a fatty acid, sugar, protein, and hormone, and can specifically pass macromolecules, such as electrolytes, such as water, sodium ion, and a chlorine ion, a fatty acid, sugar, protein, and hormone. Protein, such as electrolytes, such as water, sodium ion, and a chlorine ion, a fatty acid, sugar, and cytokine, hormone, etc. have the preferably desirable film which can be passed and a part of both cell or one cell can project in the shape of a projection. Moreover, maintenance, survival, differentiation and maturation, although self-renewal is carried out, as long as a stromata cell can maintain and survive and CD34 positivity cell does not check a membranous material at all, it may be what kind of material. As a material, polyethylene terephthalate, a polycarbonate, etc. specifically mention, and it is ****. Furthermore, the film may be the configuration of the arbitration which changes without a configuration's being fixed, and a configuration's may be fixed. The configuration of the arbitration which changes without the configuration and globular form by which the plane, the semi-sphere, and a part like a tank are opened wide, the shape of a tube, and a configuration being specifically fixed is sufficient. At this time, in the case of the film of closed systems (sealing system), such as a globular form, a desired cell is put into the interior of membranous, and it puts the 2nd cell into a membranous outside, or puts the 2nd cell into the interior of membranous conversely, and should just put a desired cell into a membranous outside. Moreover, membranous hardness may be what kind of thing. The film may use support if needed. A stromata cell can maintain and survive as support at this time, and maintenance, survival, differentiation and maturation, although self-renewal is carried out, as long as CD34 positivity cell does not prevent at all, the thing of what kind of material and configuration may be used. As a material of susceptor, glass, synthetic resin, natural resin, or a metal specifically mentions, and it is ****.

[0041] In transplanting a desired cell (specifically CD34 positivity cell) Since an immunoreaction may occur in the living body of the recipient after growth when using another individual and the stock-ized cell, even if it is

urns out that CD34 positivity cell with which the experiment was presented is normal CD34 positivity cell ıopulation (R1 field of drawing 5) which contains neither the cell which carried out destructive extinction, in ıiew of the magnitude (FSC) of the cell, and the value (SSC) of a consistency, nor CD shade sexual cell. Moreover, according to drawing 6 , the FITC count of the fluorescence intensity of CD34 of the cord blood origin CD34 positivity cell contained to R1 field of drawing 5 was the ensemble of 40 thru/or 500 as a result of FACSort analysis, and the manifestation of CD33 molecule in this cell population was the ensemble of 10 thru/or 000 at PE count. Next, in order to investigate the effect of cytokine, CD34 positivity cell separated from the ıbove-mentioned cord blood was cultivated for ten days under existence of interleukin 3 (rh-IL -3) and a stem ;ell factor (rh-SCF), and the property distribution over the relation of the FSC/SSC distribution and anti-CD34 ıntibody / anti-CD33 antibody was investigated like the above. The result is shown in drawing 7 and drawing 8 . \ccording to drawing 7 , it is suggested that the inclination which is large as a whole was seen, and fission and lifferentiation of a cell were guided by cytokine rather than them (refer to drawing 5) of CD34 positivity cell vhich just separated FSC (magnitude of a cell) and SSC (consistency of a cell) of the whole blood spherocyte ıfter cultivating for ten days by rh-IL -3 and rh-SCF from cord blood. Although drawing 8 is drawing showing the ·esult of having measured the fluorescence intensity of FITC and PE about the corpuscle cell fraction contained .o R1 field (corpuscle cell field) of drawing 7 , according to this, it turns out that the FITC count after culture is ·alling to 80 from 4 to the FITC count of CD34 positivity cell just separated from cord blood having been the ;nsemble of 40 thru/or 500. Moreover, according to the report of various reference, this was supported by this ;xperiment, although specializing in CD34 shade sexual cell was known when it cultivated by in vitro that the ıative fluorescence of CD34 shade sexual cell after culture increased [many of] from the time of the just ;eparated cord blood CD34 positivity cell, i.e., CD34 positivity cells. For this reason, a little less than 34 CD ›ositivity cell in CD34 positivity cell after culture was able to consider possibility that fluorescence intensity vould overlap CD34 shade sexual cell. Then, especially the manifestation of CD34 molecule was high and it was ised for the analysis after using as 34 or so CD positivity cell CD34 positivity cell contained to the field (R3 ield) accepted as a clearly different ensemble from CD34 shade sexual cell. Although R4 in drawing fields were CD34 negative thru/or a weak positivity cell population, according to our examination, the ratio of a little less :han 34 CD positivity cell closed to R4 field (drawing 8 and below-mentioned drawing 23) was very small. Moreover, when it cocultivates with a stromata cell, a stromata cell is usually contained to R2 field. The number ›f 34 or so CD positivity cells actually counted henceforth comparatively therefore,(%) By multiplying by the ·alue which broke the rate of the cell contained to R3 and R4 each field in the value with which all the rates of :he cell contained to(%) to R2, R3, and R4 each field in [applying only the cell of R3 field] were **(ed) and ıınited by the value with which it **(ed) and united comparatively After excepting the value of the stromata cell nixed on FACSort and multiplying this value by the number of whole blood spherocytes, it carried out by dividing ›y 100. That is, the formula of the rate that the number of cells in actual R3 field occupies is as follows.

R3(%) x(R2+R3+R4)/on actual R3(%) = appearance (R3+R4)

[0049] It is 10-6M in each well of 24 well tissue culture plate (product made from Falcon) about an example 5. ;ord blood CD34 positivity cell and CD34 positivity cell (5x103 cell / ml) of the cocultivation cord blood origin of ı mouse stromata cell strain. Hydro cortisone the added MIERO cult H5100 (12.5%HS and 12.5% new-born calf ;erum (FCS) —) 10-4M 1ml was added and alpha-MEM:STEMCELL Technologies Inc . company make which ıdded 2-mercaptoethanol was further cocultivated with the mouse stromata cell strain under existence of ;ytokine (rh-IL -3 and SCF) (contact culture). Ten days after cultivating, pipetting was fully performed, cells vere collected, by the nylon mesh, after filtration, centrifugal separation was carried out and the cultured cell vas extracted. Immuno full ORESSENSU dyeing was performed for the obtained cell, and the cell surface marker vas measured by flow cytometry (FACSort). In addition, it experimented similarly about the case where a mouse ;tromata cell strain is not used for a comparison. A result is shown in Table 1.

[0050]

[Table 1]

表 1

サイトカイン存在下における各ストローマ細胞の全血球細胞及びCD34強陽性細胞増殖効果

ストローマ細胞及びサイトカイン (IL-3+SCF)	0日	7日		10日	
	投入CD34陽性細胞	全血球細胞数 (増加倍率)	CD34強陽性細胞数 (増加倍率)	全血球細胞数 (増加倍率)	CD34強陽性細胞数 (増加倍率)
ストローマ細胞無	5000	12.6×10^4 (25.2)	2730 (0.55)	72.4×10^4 (145)	3040 (0.61)
HESS-1	5000	5.2×10^4 (10.4)	8710 (1.7)	25.2×10^4 (50)	9500 (1.9)
HESS-5	5000	38.6×10^4 (77.2)	29900 (6.0)	138.4×10^4 (277)	44400 (8.9)
SSXL CL.3	5000	17.2×10^4 (34.4)	7830 (1.6)	87.2×10^4 (174)	14500 (2.9)
SSXL CL.7	5000	4.6×10^4 (9.2)	4140 (0.83)	25.6×10^4 (51)	6170 (1.2)
SSXL CL.9	5000	8.0×10^4 (16.0)	6120 (1.2)	22.4×10^4 (45)	3490 (0.70)
SSXL CL.17	5000	5.6×10^4 (11.2)	5660 (1.1)	18.0×10^4 (36)	5310 (1.1)

0051] Here, the supplied cell is CD34 positivity cell and it is 34 or so CD positivity cell among these CD34 positivity cell about 70% of statistically. Therefore, although the increment scale factor of 34 or so CD positivity cell of Table 1 has shown the numeric value simply divided by the number (5000) of injection cells, an actual increment scale factor is the value broken by the statistical numeric value (3500), and the numeric value of an increment scale factor goes up further. According to Table 1, although there is a difference of extent regardless of the existence of a stromata cell strain, by adding cytokine shows that a whole blood spherocyte increases. However, if a stromata cell does not exist about 34 or so CD positivity cell, even if it adds cytokine, it will decrease. Furthermore, in growth of a whole blood spherocyte, HESS-5 cell showed the best effectiveness under a stromata cell strain and cytokine existence, the increment scale factor of the number of whole blood spherocytes became 70 or more times after the injection in the 7th day, and it turned out after the injection that the increment scale factor of the number of whole blood spherocytes becomes 270 or more times in the 10th day. Furthermore about the number of 34 or so CD positivity cells, the effectiveness also in a stromata cell strain that HESS-5 cell was the highest was shown, and, as for the increment scale factor in the 10th day was found by increasing about 9 times after about 6 times and an injection after the injection in the 7th day. Moreover, when the gap of the increment scale factor of 34 or so CD positivity cell by the class of stromata cell was seen, it turned out after the injection that HESS-5, SSXL CL.3, HESS-1, SSXL CL.7, SSXL CL.17, and the effectiveness as which the order of SSXL CL.9 is sufficient are shown in the 10th day. Here, especially HESS-5 that showed the good growth effectiveness had international deposition number FERM BP-5768, and they performed international deposition to National Institute of Bioscience and Human-Technology, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry, on December 6, 1996. Although these CD34 positivity cell makes the number of whole blood spherocytes increase when it cultivates only by cytokine by the above thing, and the number of pluripotency hematopoietic stem cells

34 or so CD positivity cell) used as a hematogenous source is in the inclination which decreases gradually and s finally drained, when the stromata cell of HESS-5 grade is made to live together, it turns out that these ɔluripotency hematopoietic stem cell carries out self-multiplication, and increases remarkably rather.
0052] The effectiveness cord blood CD34 positivity cell (5x103 cells) of cytokine (rh-SCF and rh-IL -3) and ıydro cortisone exerted on the cord blood CD34 positivity cell by the example 6. mouse hematogenous support ;tromata cell strain (HESS-5) To each well of 24 well tissue culture plate (product made from Falcon) Under 50 ıg(s)/a ml SCF and 20 ng(s)/ml coexistence of IL-3, or un-living together, Moreover, by cocultivating with ·IESS-5 in MIERO cult H5100(product made from Stem Cell) 1ml under the monograph affair under coexistence ɔf the hydro cortisone of 10-6M, or un-living together (contact culture) The effectiveness of cytokine (rh-SCF ınd rh-IL -3) and hydro cortisone was examined. Ten days after cultivating, cells were collected, immuno full)RESSENSU dyeing was performed, and the cell surface marker was measured in flow cytometry (FACSort). The result of the increment of the result of the increment in the number of whole blood spherocytes in 34 or so CD positivity cell to drawing 9 was shown in drawing 10. According to drawing 9 , the numbers of whole blood ;pherocytes are about 4x105 pieces / well irrespective of addition of hydro cortisone, and un-adding under ;ytokine nonexistence, and it became clear that there is no effectiveness of hydro cortisone. When cytokine was ıdded, the number of whole blood spherocytes increased remarkably irrespective of hydro cortisone addition and ın-adding, and when hydro cortisone especially existed, the rise was carried out 1.5 times [bottom / of ıonexistence] (1.2x106 receive 1.8x106). According to drawing 10, the number of CD34 positivity cells when the ıumber of 34 or so CD positivity cells adds hydro cortisone under cytokine nonexistence was set to 6x104, and t was about 12 times the basis, in [hydro cortisone] not adding, was set to 1.3x105, and similarly was about 26 ;imes the basis, and the conditions of the growth effectiveness over 34 or so CD positivity cell of not adding [of ıydro cortisone] were higher. On the other hand under cytokine existence, the number of 34 or so CD positivity ;ells in about 22 times of a basis and **** addition increased the number of CD34 positivity cells at the time of ıdding hydro cortisone by about 68 times the basis. The above thing shows that hydro cortisone un-adding is ıighest as for the growth effectiveness under cytokine existence about growth of 34 or so CD positivity cell.
0053] The effect cord blood CD34 positivity cell of the injection CD34 positivity cell density exerted on the growth of a 34 or so HESS-CD positivity cell in contact cocultivation of 5 and a cord blood CD34 positivity cell ınder existence of example 7.rh-SCF and rh-IL -3 To each well of 12 well culture plate, in MIERO cult H5100 (product made from Stem Cell; 12.5%HS and 12.5% new-born calf serum (FCS)) 2ml Seeding was carried out so ;hat HESS-5 might become the consistency of 1x103, 3x103, 1x104, and 3x104 cells into 1 well (4cm2) under ;ontact and a non-contact condition, and it cultivated for ten days. The result of the increment scale factor of a whole blood spherocyte was shown in drawing 11, and the result of the increment scale factor of 34 or so CD ɔositivity cell was shown in drawing 12. the time of the number of injection cells of the growth scale factor of a whole blood spherocyte being 1000 pieces under the conditions in which HESS-5 do not exist according to drawing 11 -- about about 380 times -- it is -- said -- the time of being 3000 pieces -- 350 times -- said -- ;he time of 10000 pieces -- about 300 times -- said -- it was about about 200 times at the time of 30000 ɔieces, and the increment scale factor of the number of whole blood spherocytes improved a little, so that there was little injection CD34 positivity cell density. On the other hand, when HESS-5 existed (contact), when about 1050 times, about 800 times, about 380 times, and about 150 times were shown and the stromata cell (HESS-5) existed, the increment scale factor of the growth scale factor of the number of whole blood spherocytes of the ıumber of whole blood spherocytes improved remarkably, respectively. Moreover, under existence of HESS-5, ;he increment scale factor of the number of whole blood spherocytes improved remarkably, so that there was ittle injection CD34 positivity cell density. According to drawing 12, the increment scale factor of a 34 or so HESS-CD positivity cell in case 5 does not exist Although only the single digit increment scale factor was shown also when the number of injection cells was any, if HESS-5 exist When the number of injection cells was 1000 ɔieces, it turned out to being about 60 times the growth scale factor of 34 or so CD positivity cell of this that the growth scale factor of 34 or so CD positivity cell is about about 10 times at the time of said 30000 individuals. When the 10000 number of this to injection cells was 1000 pieces to 3000 conditions preferably from 1000 pieces and made a stromata cell (HESS-5) live together, it became clear that the increment scale factor of CD34 positivity cell improves remarkably. It is shown that the above result will cause decline in growth effectiveness if CD34 positivity cell density at the time of an injection is too high, it is sufficient 2 well 4cm, and 1000 to 3000 cell density is especially considered to be desirable.
[0054] In order to observe the growth situation of an example 8. half-solid methyl cellulose analysis hematopoietic stem cell and a precursor cell, Methocult GF H4434V [of marketing] (Stemcell Technology Inc. -

· shrine make --) of a half-solid 0.9%Iscove methyl cellulose, 30%FCS, 1%BSA, The 2-mercaptoethanol of 10-4M, he L-glutamine of 2mM, The colony organization potency of a cord blood origin CD34 positivity cell was ıeasured using the culture medium containing 3U/ml erythropoietin, 50 ng/a ml rh-SCF, 10 ng/a ml rh-G-CSF, nd 10 ng(s)/ml rh-IL -3. First, after cultivating CD34 positivity cell separated from cord blood for ten days ınder existence of a stromata cell (HESS-5) or nonexistence in MIERO cult H5100 culture medium using a ulture dish with a diameter of 35mm, this cell was moved to the dish with a diameter of 35mm filled up with the Aethocult GF H4434V above-mentioned culture medium, and it incubated at 37 degrees C for 21 days in the air vhich contains a carbon dioxide 5%. On the other hand, this was cultivated with direct above-mentioned Aethocult GF H4434V culture medium, without cultivating CD34 positivity cell separated from cord blood as ontrast with MIERO cult culture medium. The number of BFU-E formed into Methocult GF H4434V culture ıedium, the erythron colony which consists of CFU-GEMM, a CFU-GM colony, and HPP-CFC colonies was ıeasured. HPP-CFC is the colony of magnitude with a diameter of 1.0mm or more, and calculated it as large-ized HPP-CFC with still larger small HPP-CFC of a 1.0 to 2.5 mm diameter and diameter than 2.5mm. the result f the increment scale factor of the colony count of large-sized HPP-CFC under [various] conditions (a liameter is larger than 2.5mm) -- drawing 13 -- the result of the increment scale factor of the colony count of CFU-GM is shown in drawing 15, and the result of the increment scale factor of the colony count of an erythron olony is shown for the result of the increment scale factor of the colony count of small HPP-CFC (a diameter s 1.0 to 2.5 mm) in drawing 14 at drawing 16. According to drawing 13, in the increment scale factor of the olony count of large-sized HPP-CFC, the increment effectiveness under existence of a stromata cell more emarkable than the bottom of nonexistence was seen. On the other hand, unless the stromata cell existed, the ıcrement scale factor of the colony count of an injection cell was low, and the significant difference between ach injection CD34 positivity cell density was not accepted. Moreover, under existence of a stromata cell, by 000 to 3000 pieces, although, as for the difference with the as significant increment scale factor of a colony ount as 24 to 26 times, the number of injection cells was not seen, the increment scale factor of 13 times, 7 imes, and a colony count fell respectively as the number of injection cells increased from 10000 pieces with 0000 pieces. Injection CD34 positivity cell density was judged that 3000 pieces are suitable from per [1000] wo 4cm also about the increment in the colony count of this to large-sized HPP-CFC. According to drawing 14, he inclination same also about the increment scale factor of the number of small HPP-CFC as the increment cale factor of the number of large-sized HPP-CFC of drawing 13 was shown. However, it was 32 to 36 times he increment scale factor at the time of optimal CD[initial] 34 positivity cell density 1000 to 3000 pieces of his, and it was higher than the increment scale factor of large-sized HPP-CFC. Although the direction under xistence of a stromata cell had remarkable effectiveness in the increment scale factor of a colony count in the ncrement in CFU-GM rather than the bottom of nonexistence, large-sized HPP-CFC and small HPP-CFC had in / to being about 20 times the increment scale factor under stromata cell existence of this as compared with t under nonexistence / CFU-GM] injection CD34 positivity cell density as low as about 3.5 times according to drawing 15. Moreover, 3000 pieces are the optimal [the effectiveness exerted on the increment in CFU-GM of nitial CD34 positivity cell density] from per [1000] two 4cm under stromata cell existence like the case of arge-sized HPP-CFC or small HPP-CFC, and, as for the growth scale factor at that time, 600 to 650 times vere shown. According to drawing 16, the increment scale factor of the erythron colony containing BFU-E and CFU-GEMM showed the effectiveness under existence of a stromata cell more remarkable than the bottom of ıonexistence. Moreover, under existence of a stromata cell, when the number of injection cells was 3000 pieces, he increment scale factor was the highest, about 46 times were shown, and when the number of injection cells vas 1000 pieces, they were about 27 times. The increment scale factor fell as the number of injection cells was ncreased with 10000 or 30000 pieces. When making the number of injection CD34 positivity cells into 3000 per wo 4cm on the conditions in contact with a stromata cell from this, it became clear that an increment scale actor is the highest.

[0055] The culture CD34 positivity cell (3x103 cells / ml) of the effectiveness mouse stromata cell strain of the ytokine (rh-SCF and rh-IL -3) exerted on 34 or so CD positivity cell proliferation in an example 9. optimum ulture condition, and a stromata cell (HESS-5), and a cord blood CD34 positivity cell the inside of each well of 12 well tissue culture plate (product made from Falcon) -- the 2ml MIERO cult H5100 (12.5%HS and 12.5% new-orn calf serum (FCS) --) The alpha-MEM:STEMCELL Technologies Inc . company make which added 10-4M 2-ıercaptoethanol is added. Furthermore, it cocultivated under existence of HESS-5 cell strain or nonexistence ınder existence of cytokine (rh-SCF and rh-IL -3) or nonexistence. Ten days after cultivating, pipetting was ully performed and cells were collected, and by the nylon mesh, centrifugal separation was carried out after

iltration and it extracted. Immuno full ORESSENSU dyeing was performed for the obtained cell, and the cell surface marker was measured by flow cytometry (FACSort). Furthermore, colony organization potency was measured by the above-mentioned methyl cellulose assay of a half-solid. The analysis result by FACSort of SSC and FSC of CD34 positivity cell cultivated for ten days under existence of cytokine and a stromata cell to drawing 17 was shown. Except that according to drawing 17 many of numbers of corpuscle cells are included by R1 field and HESS-5 do not exist, many numbers of cells which belong to R1 field clearly even if it compares with the result of drawing 7 cultivated on the same conditions exist. It became clear that many cells which show the same magnitude as CD34 positivity cell just separated from the cord blood rather shown by drawing 5 and a consistency exist. Moreover, the cell of HESS-5 and its fragment were observed at places other than R1 field. It is CD34 positivity cell cultivated for ten days under existence of cytokine and a stromata cell to drawing 18 , and the analysis result by FACSort when dyeing the corpuscle cell belonging to R1 field of drawing 17 by FITC indicator CD34 antibody and PE indicator CD33 antibody was shown. Except that drawing 18 and HESS-5 do not exist, if drawing 8 cultivated on the same conditions is compared, it is clear that many cells exist in R3 field to which 34 or so CD positivity cell exists in drawing 8 . According to this, as shown in drawing 8 , when most of CD34 positivity cells, especially strong positivity cells specialize and mature, only in the case of cytokine, it will be exhausted, but when [with a stromata cell] it cocultivates, it turns out that continuation maintenance of the 34 or so CD positivity cell (pluripotency hematopoietic stem cell) which has self-renewal ability is still carried out. The analysis result by FACSort of SSC and FSC in CD34 positivity cell cultivated for ten days under existence of a stromata cell under the nonexistence of cytokine to drawing 19 was shown. According to drawing 19 , like the case of drawing 17 , cell distribution of the R1 field is similar with the pattern of CD34 positivity cell just separated from the cord blood shown by drawing 5 , and has become things fewer than the time of also cultivating the cell (FSC becoming large since the cell of a mitotic phase becomes large) of a mitotic phase under cytokine existence and stromata cell nonexistence. The analysis result by FACSort when dyeing by FITC indicator CD34 antibody of a corpuscle cell and PE indicator CD33 antibody which are contained in drawing 20 to R1 field among CD34 positivity cells cultivated for ten days under existence of a stromata cell under the nonexistence of cytokine was shown. According to drawing 20 , there were most ratios of 34 or so CD positivity cell contained to R3 field in other experimental groups. Moreover, the result of the number of whole blood spherocytes under [various / by the above-mentioned experiment actuation] a culture condition and the number of 34 or so CD positivity cells was shown in Table 2.

[0056]

[Table 2]

表 2

全血球細胞、CD34強陽性細胞におけるサイトカイン及びHESS-5細胞の増殖効果

条件	全血球細胞数（個／ウェル）	全血球細胞数の倍率（倍）	CD34強陽性細胞数の比率（%）	CD34強陽性細胞数（個／ウェル）	CD34強陽性細胞数の増加倍率（倍）
0日 投入CD34陽性細胞数	3000		79.7±8.4	2390±250	
10日 サイトカイン無 HESS-5細胞無	3600±1250	1.2±0.4	23.3±3.6	840±130	0.4±0.1
10日 rh-IL-3+rh-SCF HESS-5細胞無	944000±151000	315.0±50.3	0.5±0.2	4720±1890	2.0±0.8
10日 サイトカイン無 HESS-5細胞存在下	63100±12300	21.0±4.1	23.2±4.8	14600±3030	6.1±1.3
10日 rh-IL-3+rh-SCF HESS-5細胞存在下	2544000±499000	848.0±166.0	7.0±1.4	178000±35600	74.0±14.9

[0057] According to Table 2, although the number of whole blood spherocytes was seldom as changeful as 1.2 times when a cord blood CD34 positivity cell was cultivated only with MIERO cult H5100 culture medium, in order that the rate of 34 or so CD positivity cell might decrease remarkably, the calculated number of 34 or so CD positivity cells also decreased by 0.4 times. In the culture in which rh-IL -3 and rh-SCF exist, and HESS-5 do not exist on the other hand, although it increased **50.3 times, the increment rates of 34 or so CD positivity cell were few, and the calculated increment in the number of 34 or so 315CD positivity cells of the number of whole blood spherocytes was about 2.0 times. On the other hand, when a cord blood CD34 positivity cell was cultivated under existence of HESS-5 cell under cytokine nonexistence, the whole blood spherocyte increased 21.0**4.1 times from the number of start cells. Although this increment scale factor is low as compared with the culture which added only cytokine, since 34 or so CD positivity cell was included 23.2**4.8%, the number of culture of 34 or so CD positivity cell was increasing by 6.1**1.3 times, and it became clear that HESS-5 are very effective in the self-multiplication of 34 or so CD positivity cell. When cytokine was furthermore added under this culture condition, the number of whole blood spherocytes increased from the time (315**50.3 times)

if HESS-5 cell not existing further (848**166 times). To the case of only cytokine being twice [about], the number of 34 or so CD positivity cells became 74 times, when cytokine and HESS-5 lived together, and it rose dramatically with both combination. Furthermore, the result of the number of colony forming cells under various culture conditions was shown in Table 3.

0058]

Table 3]

表 3

各コロニーにおけるサイトカイン及びHESS-5の増殖効果

条件	コロニー数（個／ウェル）			
	HPP-CFC(>2.5mm)*	HPP-CFC(1-2.5mm)*	CFU-GM	赤血球系コロニー
0日 投入CD34陽性細胞数	192±40	240±50	300±35	390±60
10日 サイトカイン無 HESS-5細胞無	14±6	24±16	119±16	36±7
10日 rh-IL-3+rh-SCF HESS-5細胞無	280±170	480±160	54100±14100	800±690
10日 サイトカイン無 HESS-5細胞存在下	640±160	4020±420	7500±840	540±280
10日 rh-IL-3+rh-SCF HESS-5細胞存在下	8420±810	11400±1110	187165±14100	17700±5000

*HPP-CFC(>2.5mm)は、HPP-CFCコロニーの直径が2.5mmより大きいコロニーを示し、
HPP-CFC(1-2.5mm)は、HPP-CFCコロニーの直径が1mmから2.5mmであるコロニーを示す。

0059] When a cord blood CD34 positivity cell was cultivated only by the MIERO cult H5100, all the colony counts decreased remarkably as compared with the time of an injection. On the other hand, although CFU-GM is remarkable and the increment in the precursor cell colony count under existence of cytokine (rh-IL -3 and rh-SCF) increased as compared with the time of an injection (300**35) (54100**14100, about 180 times), small HPP-CFC (one to 2.5 mm) and an erythrocyte precursor cell were as compared with the time of an injection 390**60) as twice [about] (800**690). Large-sized HPP-CFC (> 2.5mm) remained in about (at the time of an injection 192 after [culture] from **40 280 **170) about 1.5 times. On the other hand, when cultivated under the conditions in which a stromata cell (HESS-5) exists under cytokine nonexistence, the CFU-GM colony count 7500**840) was extent which is about 25 times increasing extent of this, and an erythron colony count 540**280) also increases from it at the time of an injection (300**35) by about 1.4 times rather than it at the time of an injection (300**35). The small HPP-CFC (one to 2.5 mm) colony count (at the time of an injection 240 **50, after [culture] 4020 **420) increased more remarkably than the time of the conditions in which only about 17 times and cytokine exist. The large-sized HPP-CFC (> 2.5mm) colony count (540**160) also increased from the time of the conditions in which only cytokine exists (from about 1.5 times to about 3.3 times). Moreover, under the conditions in which a stromata cell (HESS-5) and cytokine (rh-IL -3 and rh-SCF) exist, the large-sized HPP-CFC colony count (8420**810) and the small HPP-CFC colony count (11400**1110) increased dramatically with about 44 times and about 48 times as compared with it at the time of an injection (respectively 192 **40,240 **50), respectively. The colony count (187165**14100) of CFU-GM also increased from under the condition in which only cytokine exists (about 180 times) further, and it was it. [of this] [about 620 times] Although the erythron colony count did not carry out the increment in a deer only in other culture conditions, it increased remarkably under the conditions in which HESS-5, rh-IL -3, and rh-SCF exist (at the time of an injection 390 after [culture] from **60 17700 **500, about 44 times). The above result shows that a HPP-CFC colony increases irrespective of the existence of the existence of cytokine, if a stromata cell (HESS-5) exists. Any colony is known by increasing dramatically, if a stromata cell (HESS-5) furthermore exists and cytokine (rh-IL -3 and rh-SCF) exists.

0060] CD34 positivity cell separated from the cell surface marker cord blood of re-separated CD34 positivity

cell after example 10. magnification, After cultivating it under the conditions in which the above-mentioned stromata cell (HESS-5) and cytokine (rh-IL -3 and rh-SCF) exist out of a living body, From the amplified whole blood spherocyte, CD34 positivity cell They are CD34 pro JIENITA isolation kit (product made from Progenitor solation Kit (QBend/10)), and a MACS-magnetic cell-sorting system () again. [Magnetic Cell] Sorting System Miltenyi Biotec GmbH) It re-dissociated using shrine make. The purity of re-separated CD34 positivity cell was 97% or more. The amount of manifestations of CD34, CD33, CD38, and CD13 was analyzed in FACSort using anti-CD34 antibody, anti-CD33 antibody, anti-CD38 antibody, and alpha anti-D 13 antibody after that. The analysis result of FSC and SSC of the just separated cord blood CD34 positivity cell and after [magnification] re-separated CD34 positivity cell was shown in drawing 21 and drawing 22 , respectively. Since re-separated CD34 positivity cell is larger than that of the cord blood CD34 positivity cell which magnitude and a consistency just separated, it is predicted that the cell has shifted to the mitotic phase. Then, various cell surface markers were analyzed about the cell contained to a corpuscle cell field (drawing 21 or R1 field of drawing 22). The dyeing pattern of CD34 and CD38 was shown in drawing 23 and drawing 24 , and the dyeing pattern of CD34 and CD13 was shown for the dyeing pattern of CD34 and CD33 of a cord blood CD34 positivity cell and after [magnification] re-separated CD34 positivity cell in drawing 27 and drawing 28 as well as drawing 25 and drawing 26, respectively. The rate of CD33 positivity cell in re-separated CD34 positivity cell (drawing 24) increased from separated fresh CD34 positivity cell (drawing 23) clearly (they are 73.9(10.1+63.8) % and 93.6 (17.2+76.4) %, respectively). This means that CFU-GEMM, CFU-GM, EO-CFC, and BFU-E are increasing more as compared with CD34 positivity cell with re-separated fresh CD34 positivity cell. However, the thing of re-separation should note that 34 or so CD positivity cell field is increasing remarkably. The rate of the 38 or so 34 or so CD positivity CD positivity cell was also increasing remarkably in the re-separation CD34 positivity cell after in vitro magnification, and, as for the rate (3.5%) of the 34 or so CD positivity CD38 low positivity / shade sexual cell ensemble in re-separated CD34 positivity cell, cord blood was decreasing comparatively (6.2%) so that clearly from drawing 25 and drawing 26 . However, if it saw from a 34 or so CD positivity CD38 low positivity / the number of shade sexual cells, since 34 or so CD positivity cell would increase 74 times after magnification (refer to Table 2), it became clear that the 34 or so CD positivity CD38 low positivity / the number of shade sexual cells is 42 (74-x-3.5/6.2)-double-increasing by magnification. This increment scale factor was well [as the increment scale factor of the number of HPP-CFC] in agreement. According to drawing 27 and drawing 28 , the 34 or so CD positivity CD13 low positivities / shade sexual cell ensembles of a cord blood CD34 positivity cell were increasing in number conversely as compared with the manifestation of CD33 and CD38 antigen (respectively 12.4%, 16.1%). Since the whole number of cells increases and the rate is also increasing, it turns out that the cell before determining differentiation of the 34 or so CD positivity / CD13 low positivity / the number of shade sexual cells, i.e., a bone marrow system cell, is increasing. As for the rate of 34 or so CD positivity cell in a cord blood CD34 positivity cell, and a CD34 low positivity cell, after growth was not seen, as for change (they are 79.7(63.8+15.9) % and 79.5(76.43.1) %, more nearly respectively than drawing 23 and drawing 24). Although it had reported that 34 or so CD positivity cell was contained 22% in CD34 positivity cell, and Broxmeyer and others showed the property of a very undifferentiated cell, when FACSort was analyzed on the same level as this, these 34 or so CD positivity cell was contained at a very high rate in re-separated CD34 positivity cell after in vitro magnification (55.0%).

[0061] CD34 positivity cell separated from the analysis cord blood of the cell cycle of CD34 positivity cell before and behind example 11. magnification Under existence of the cytokine (rh-IL -3 and rh-SCF) above-mentioned in the outside of a living body, After cocultivating with a stromata cell (HESS-5), From the amplified whole blood spherocyte, CD34 positivity cell They are CD34 pro JIENITA isolation kit (product made from Progenitor Isolation Kit (QBend/10)), and a MACS-magnetic cell-sorting system () again. [Magnetic Cell] Sorting System (MiltenyiBiotec GmbH) It re-dissociated using shrine make. Thus, the erythrocyte of a fowl and the nucleus of thymocyte were considered for the obtained magnification CD34 positivity cell and CD34 positivity cell just separated from cord blood as control, and the rate of the cell cycle of each phase of a G0-/G plane 1, an S phase, and a G2 / M phase was analyzed using CellFIT software (Becton Dickinson) attached to FACSort. The analysis result of the cell cycle of a cord blood CD34 positivity cell and after [magnification] re-separated CD34 positivity cell was shown in drawing 29 and drawing 30, respectively. Generally it was known that a growth phase is a period between S- and G2-/mitotic phases, and only only 2.6(S phase 1.3+G2-/M phase 1.3) % of CD34 positivity cell just separated from cord blood was a growth phase. On the other hand, in after [magnification] re-separated CD34 positivity cell, 41.6(S phase 16.6+G2-/M phase 25.0) % exists in the growth phase, and this result shows that the culture under existence of cytokine and a stromata cell promotes the cell

ycle of a cord blood CD34 positivity cell with many cells of a resting phase to S or G2-/mitotic phase about
:D34 positivity cell.
0062] Seeding of the effectiveness cord blood CD34 positivity cell of HESS-5 exerted on growth of an example 2. cord blood CD34 positivity cell and various cytokine was carried out so that HESS-5 might become the onsistency of 3x103 cells into 1 well (4cm2) under contact and a non-contact condition at each well of 12 well ulture plate into MIERO cult H5100(product made from Stem Cell 12.5% HS and 12.5% new-born calf serum FCS)) 2ml. Next, it added to the well and the Homo sapiens cytokine of various concentration was cultivated for en days. The last concentration of each cytokine was a flk2L, 100 ng/ml MIP-1alpha, 20 ng/ml G-CSF, and 1U/ 20 ng/ml IL-3 and 50 ng/ml SCF, and 50 ng/ml) ml EPO and 20 ng/ml GM-CSF. Cells were collected ten days fter culture and the cell surface marker was measured by flow cytometry (FACSort). A result is shown in Table .

0063]
Table 4]

表 4

全血球細胞、CD34強陽性細胞における各種サイトカイン及びHESS-5細胞の増殖効果

サイトカイン	全血球細胞数の増加倍率（倍）		CD34強陽性細胞の増加倍率（倍）	
	w/o HESS-5*	w HESS-5*	w/o HESS-5*	w HESS-5*
none	1.0	6.7	0.1	2.8
IL-3	20.7	133	1.1	29.0
SCF	9.3	110	0.2	26.3
IL-3+SCF	70.0	256	1.5	44.0
IL-3+SCF+flk2 ligand	310	446	2.7	18.0
IL-3+SCF+MIP-1α	89.0	127	1.6	19.0
IL-3+SCF+G-CSF	226	366	1.9	25.0
IL-3+SCF+EPO	543	400	3.4	54.0
IL-3+SCF+G-CSF+GM-CSF	193	460	1.9	27.7

* w/o HESS-5はHESS-5細胞非存在下であり，w HESS-5細胞はHESS-5細胞存在下を示す．

0064] In addition, the increment scale factor of the number of whole blood spherocytes of the combination of he cytokine under HESS-5 existence or nonexistence and the number of 34 or so CD positivity cells was shown n Table 4. In the number of whole blood spherocytes, in any case, the number increased by existence of various ;ytokine irrespective of existence of HESS-5 and nonexistence. Moreover, regardless of the existence of ;ytokine, or the kind of situation, the increment scale factor about a whole blood spherocyte and 34 or so CD ›ositivity cell improved by existence of HESS-5 cell except for the case of IL-3+SCF+EPO conversely. Under he nonexistence of HESS-5 cell, the increment scale factor of the number of whole blood spherocytes of IL-3+SCF+EPO was the highest, showed 543 times, and was the order without IL-3+SCF+flk2L and IL-3+SCF+G-CSF, IL-3+SCF+G-CSF+GM-CSF, IL-3+SCF+MIP-1alpha, IL-3+SCF, IL-3, SCF, and cytokine in order of below. On the other hand, under the conditions in which HESS-5 exist, the increment scale factor with highest IL-3+SCF+G-CSF+GM-CSF was shown. the order of the following -- IL-3+SCFflk2L and IL-3+SCF+EPO, IL-3+SCF+G-CSF, IL-3+SCF, IL-3, and IL-3+SCF+MIP -1 -- it was order without alpha, SCF, and cytokine. About he increment scale factor of the number of 34 or so CD positivity cells, remarkable growth was accepted in neither of the combination of a maximum of 3.4 times and the cytokine from 0.1 times under the conditions in which HESS-5 do not exist. On the other hand, compared with the increment scale factor of 34 or so CD

ositivity cell, the growth effectiveness of 10 times or more improved remarkably by combining various cytokine ith the bottom of HESS-5. About the combination of cytokine, IL-3+SCF+EPO was 54 times most highly. It was following] the order of IL-3+SCF, IL-3, and IL-3+SCF+G-CSF+GM-CSF, SCF, IL-3+SCF+G-CSF, IL-+SCF+MIP-1alpha, IL-3+SCF+flk2L, and cytokine nothing in order of.

0065] Contact cocultivation of an example 13. cord blood CD34 positivity cell and HESS-5 cell, The indirect ontact culture CD34 positivity cell (5x103 cell / well) of non-contact cocultivation of a cord blood CD34 ositivity cell and HESS-5 cell and a cord blood CD34 positivity cell, and HESS-5 cell in each well of 6 well issue culture plate (product made from Falcon) The 4ml MIERO cult H5100 (12.5%HS and 12.5% new-born calf erum (FCS)) which added 50 ng(s)/a ml SCF and IL-3 [20 ng(s)/ml], It cultivated under the nonexistence of IESS-5, or existence of HESS-5 in [by the alpha-MEM:STEMCELL Technologies Inc . company] having added he 2-mercaptoethanol of 10-4M. It cultivated in the state of the indirect contact which minded the mere ontact condition, the non-contact condition through a base material, and the base material as cocultivation onditions for HESS-5 and CD34 positivity cell as illustrated to drawing 2 , drawing 3 (a), and drawing 4 (a), espectively. Cocultivation in the contact condition illustrated by drawing 2 was carried out by carrying out eeding of the HESS-5 cell on 6 well plate, and carrying out seeding of the CD34 positivity cell to the shape of a ayer on the HESS-5 cell further. Moreover, the culture in the non-contact condition illustrate by drawing 3 (a) arried out 5x 103-piece seeding of the CD34 positivity cell inside this base material while insert the cel culture nsertion (Cell Culture Insert) (the product made from Falcon: base material made from plastics of the silk hat onfiguration which have much micropores) which have attach the Cyclopore film (0.45 micrometers of ipertures) into 6 well plate which carried out seeding of the HESS-5 cell (refer to drawing 3 (a)). Culture in the ndirect contact condition illustrated by drawing 4 (a) was carried out as follows. In order to make HESS-5 cell nd a cord blood CD34 positivity cell cultivate on both sides of the Cyclopore film which constitutes a base naterial Into sterile 500ml beaker filled up with 200ml of alpha-MEM which added the horse blood serum (HS) 0% first, where vertical transposition is carried out, a cel culture insertion (Cell Culture Insert) (product made rom Falcon) is inserted. Multistory [of the suspension (5x105 cell / ml) 1ml of HESS-5 cell] was calmly carried ut to the top face (outside: base of a silk hat configuration base material) of **** with the pipet, and it ultivated at 37 degrees C in the air which contains a carbon dioxide 5%. The cel culture insertion to which IESS-5 were fixed to the membranous base outside was taken out from the beaker with the sterile pincettes 48 ours after culture, and it set like drawing 4 (a) into 6 well culture plate. Subsequently, seeding of the cord blood D34 positivity cell (5x103 cell / well) was carried out inside [base] the cel culture insertion, and it ocultivated on the same conditions as the above-mentioned (indirect contact culture). In addition, culture of D34 positivity cell was also performed under HESS-5 cell nonexistence as contrast. Pipetting of the cell was arried out after culture for ten days, respectively, and it filtered by the nylon mesh, and collected by performing entrifugal separation. These cells were dyed by immuno full ORESSENSU like the above-mentioned, and the ell surface marker was analyzed by flow cytometry (FACSort). The increment scale factor of a whole blood pherocyte and 34 or so CD positivity cell is shown in drawing 31 and drawing 32, respectively. According to drawing 31, the increment scale factor of the number of whole blood spherocytes showed the growth effectiveness that what was cocultivated where indirect contact of CD34 positivity cell and the HESS-5 cell is arried out by membranous both sides was remarkable like drawing 4 (a), and it was it compared with the ncrement scale factor under stromata cell nonexistence, and was about 820 times the increment scale factor of he whole blood spherocyte at this time of this. [of this] [about 2.5 times] On the other hand, compared with he growth scale factor under stromata cell nonexistence, contact culture with HESS-5 and this non-contact ulture are 1.5 times and 1.2 times, respectively, and showed the growth effectiveness of being about 270 times nany as this, under the nonexistence of about 350 times and a stromata cell by 400 times and non-contact ulture with HESS-5 by contact culture with HESS-5. According to drawing 32, by about 42 times and non-ontact culture with HESS-5, it is about 15 times under the nonexistence of the stromata cell of 34 or so CD ositivity cell, and the effectiveness of the three conditions that contact cocultivation was the highest was hown by 4 times and contact culture with HESS-5. Although there was no difference with the as big increment cale factor of the number of whole blood spherocytes as contact cocultivation, in non-contact cocultivation, it as falling to 2.8 by about 1/about the increment scale factor of the number of 34 or so CD positivity cells. On he other hand, by indirect contact cocultivation using both sides of the base material film, the increment scale actor of the number of 34 or so CD positivity cells showed the growth effectiveness almost equivalent to about 40 times and contact cocultivation. From the above thing, when cultivating CD34 positivity cell under existence of the stromata cell of HESS-5 grade, it is not necessary to not necessarily contact both cells directly, and it is

ıeard and found that both may be cultivated in the state of non-contact or indirect contact within the same ;ulture container. Especially non-contact cultivation and indirect contact cultivation are convenient, although ›oth cells are separated after culture and CD34 positivity cell is taken out, and they are greatly utility at ıtilization.

0066] About the stromata cell acquired from the bone marrow of example 14. mouse stromata cell strain HESS-8 and the preparation C3 H/HeN mouse of HESS-M28, it is alphaMEM culture medium (a horse blood serum, a leoxy nucleotide, and deoxyribonucleotide are included 10%.). Long term culture was carried out all over the ılikken biotechnology medical laboratory, and cloning was carried out with the extra dilution method. The ıbtained stromata cell substrain was cocultivated with the Homo sapiens cord blood origin CD34 positivity cell, ınd the capacity which amplifies CD34 positivity cell chose the high cell strain. The obtained cell strain was ıamed HESS-18. HESS-18 -- international deposition number FERM BP-6187 -- with, international deposition ıas carried out on November 28, 1997 in National Institute of Bioscience and Human-Technology, Agency of ıdustrial Science and Technology, the Ministry of International Trade and Industry. About this HESS-18 ;tromata cell strain, it is alphaMEM culture medium (a horse blood serum, a deoxy nucleotide, and leoxyribonucleotide are included 10%.). It cultivated for five months all over the Nikken biotechnology medical ıboratory, and cloning was carried out with the extra dilution method. The obtained stromata cell substrain was ;ocultivated with the Homo sapiens cord blood origin CD34 positivity cell, and the capacity which amplifies CD34 ıositivity cell chose the high cell strain. The obtained cell strain was named HESS-M28. HESS-M28 -- ıternational deposition number FERM BP-6186 -- with, international deposition was carried out on November !8, 1997 in National Institute of Bioscience and Human-Technology, Agency of Industrial Science and ˜echnology, the Ministry of International Trade and Industry. On the other hand, cloning also of low cell strain ıENS-M12 of the capacity which amplifies CD34 positivity cell was carried out to coincidence. In addition, the ıomo sapiens cord blood origin CD34 positivity cell used in this experiment was acquired like the example 2 and he example 3. The gestalt and cell surface antigen phenotype of CD34 positivity cell which were obtained were ınalyzed like the example 3 and the example 4. The result was shown in (a) of drawing 34 , and (b).

0067] The magnification capacity of the Homo sapiens hematopoietic stem cell which is CD34 high+CD38low/-›f mouse stromata cell strain HESS-5 (international deposition number: FERM BP-5768) which is the analysis ıbove-mentioned of the magnification ability of the Homo sapiens CD34 positivity cell of an example 15. mouse ;tromata cell strain, and was made and prepared, HESS-18 (international deposition number: FERM BP-6187), ıESS-M28 (international deposition number: FERM BP-6186), and HENS-M12 was measured as follows. culture contact culture) of said Homo sapiens cord blood CD34 positivity cell was carried out for ten days with the ;tromata cell of each above like the example 5 in the nutrition culture medium including recombination Homo ;apiens IL-3 (20 ng/ml) and recombination Homo sapiens SCF (a stem cell factor, 50 ng/ml). The property and ;otal of a CD34 high+CD38low/-cell were measured like the example 3 and the example 4 using flow cytometer ˜ACS in all the hematopoietic stem cells after culture, the CD34high+ cell, and the list. In addition, culture which ;ontains neither of the stromata cells as contrast was performed. A result is shown in drawing 35 thru/or drawing 39 . Moreover, it analyzed also about the property of each CD34+ cell of having isolated from the cell ›opulation cocultivated and obtained with HESS-5, HESS-18, and HESS-M28 using flow cytometer similarly. A ˙esult is shown in drawing 40 thru/or drawing 42 . Furthermore, the result obtained by this flow cytometry was ;valuated, and the number of cells and the computed reproductive rate were shown in Table 5.

.0068]

.Table 5]

表5

ストローマ細胞クローン	全造血幹細胞の数 ($\times 10^5$個/フラスコ)	全造血幹細胞の増幅率 (倍)	$CD34^{high+}$細胞の数 ($\times 10^5$個/フラスコ)	$CD34^{high+}$細胞の増幅率 (倍)	$CD34^{high+}CD38^{low/-}$細胞の数 ($\times 10^4$個/フラスコ)	$CD34^{high+}CD38^{low/-}$細胞の増幅率 (倍)
ストローマなし	1.2 ± 0.4	1.2 ± 0.4	0.3 ± 0.1	0.4 ± 0.1	未測定	-
マウス骨髄由来ストローマ細胞株						
HESS-5	396.0 ± 82.0	396.0 ± 82.0	74.5 ± 17.4	102.1 ± 23.1	39.6 ± 0.8	92.1 ± 1.9
HESS-18	421.3 ± 45.5	421.3 ± 45.5	103.6 ± 11.2	142.0 ± 15.3	92.7 ± 14.4	215.6 ± 23.3
HESS-18由来変異株						
HENS-M12	40.7 ± 2.2	40.7 ± 2.2	13.6 ± 0.7	18.6 ± 1.0	9.4 ± 0.5	21.7 ± 1.2
HESS-M28	409.3 ± 41.1	409.3 ± 41.1	93.7 ± 9.4	128.4 ± 12.9	143.3 ± 14.4	333.2 ± 33.4

＊培養に供したヒト臍帯血CD34陽性細胞の初期細胞数は、1×10^5個／フラスコ（その内、総$CD34^{high+}$細胞数は、73,000個、及び$CD34^{high+}CD38^{low/-}$細胞数は4,300個）。値は、平均値±ＳＤ（標準偏差）。

[0069] clear from Table 5 -- as -- HESS-5, HESS-18, and HESS-M28 -- each -- a CD34+ cell -- about 100 times -- it amplified. moreover, the undifferentiated CD34 high+CD38low/-cell by which HESS-5, HESS-18, and HESS-M28 are contained in this CD34+ cell and which is a stem cell -- 92 times as many each as this, 216 times, and 333 times -- it amplified. These values about HESS-18 and HESS-M28 are surprising values.

[0070] Analysis of the property of the Homo sapiens CD34 positivity hematopoietic stem cell cultivated and amplified under coexistence of an example 16. mouse stromata cell (1)

in the experiment of an example 15, each of mouse stromata cell strain HESS-5, HESS-18, and HESS-M28 has the capacity which amplifies intentionally a Homo sapiens CD34 positivity hematopoietic stem cell, if it puts in another way The Homo sapiens CD34 positivity hematopoietic stem cell acquired from cord blood etc. by cocultivating with either of these cell strains It was proved that a Homo sapiens CD34 positivity hematopoietic stem cell, especially the undifferentiated CD34 high+CD38low/-cell which is a stem cell could be manufactured in large quantities simple by short culture. The purpose of this experiment checks that it is CD34 positivity cell which has the same property for this natural CD34 positivity cell, without CD34 positivity cell amplified by making it such differing from fresh natural CD34 positivity cell isolated from Homo sapiens cord blood etc. in any

vay. In this experiment, the check from a viewpoint of the self-renewal ability which this CD34 high+CD38low/- :ell has, a gestalt, and a differentiation anti-original table solid pattern was performed. CD34 positivity cell was espectively isolated from the hematopoietic stem cell group manufactured by Homo sapiens cord blood and the ist in the example 5 by cocultivation with a mouse stromata cell strain (HESS-5, HESS-18, and HESS-M28) ısing the same approach as an example 2 and an example 3, i.e., CD34 Progenitor Isolation Kit, (QBend/10), and MACS Cell Sorting System (Miltenyi Biotec GmbH, Glandbach, Germany). Subsequently, the isolated CDwhich :ell sorting (cell sorting) of CD34 positivity cell population is respectively offered and carried out to FACSort low cytometer (Beckton Dickinson Immunocytometry Systems), and is undifferentiated hematopoietic stem cell 34 high+CD38low/-cell was isolated. The property of each CD34 high+CD38low/-cell population analyzed using low cytometer was shown in drawing 43 thru/or drawing 46 . In addition, the CD34 high+CD38low/-cells :ontained in a Homo sapiens cord blood CD34 positivity cell are usually about 3 thru/or 4.5% so that clearly also rom drawing 34 (b). each isolated CD34 high+CD38low/-cell (1x105 each/ml) using a culture flask (75cm2,)roduct made from Falcon) the Myelocult H5100 nutrition culture medium (30ml and 12.5% horse blood serum --) vhich added recombination Homo sapiens IL-3 (20 ng/ml) and recombination Homo sapiens SCF (50 ng/ml) Cocultivation (contact culture) was carried out for ten days with mouse stromata cell HESS-5 in the product nade from alphaMEM culture-medium;StemCell Technologies Inc. which contains fetal calf serum and the 2- nercaptoethanol of 10-4M 12.5%. Centrifugal separation was carried out, after pipetting's having recovered the :ell and filtering through a nylon mesh. The differentiation antigen analysis by the immunity dyeing test method ınd flow cytometer analyzed the property of the obtained cell. The result was shown in drawing 47 thru/or drawing 50 . Furthermore, the result obtained by this flow cytometry was evaluated, and the number of cells and :he computed reproductive rate were shown in Table 6.

[0071]

[Table 6]

表6

HESS-5との二次共培養に供したCD34^{high+}CD38$^{low/-}$細胞	全造血幹細胞の数（×10^{5}個／ウェル）	全造血幹細胞の増幅率（倍）	CD34^{high+}細胞の数（×10^{5}個／ウェル）
ヒト臍帯血から単離した新鮮なCD34^{high+}CD38$^{low/-}$細胞	15.0 ± 2.0	500 ± 67	2.5 ± 0.2
各種ストローマ細胞との一次共培養により増幅された細胞群から単離したCD34^{high+}CD38$^{low/-}$細胞			
HESS-5との一次共培養で増幅されたCD34^{high+}CD38$^{low/-}$細胞	24.6 ± 3.3	821 ± 108	3.1 ± 0.4
HESS-18との一次共培養で増幅されたCD34^{high+}CD38$^{low/-}$細胞	22.1 ± 2.1	738 ± 69	4.4 ± 0.4
HESS-M28との一次共培養で増幅されたCD34^{high+}CD38$^{low/-}$細胞	21.1 ± 2.4	702 ± 78	5.4 ± 0.6

＊値は、平均値±ＳＥ。

[0072] In an example 5, it amplifies by cocultivation (primary culture) with each of HESS-5, HESS-18, and HESS-M28 so that clearly from this drawing. Each manufactured CD34 high+CD38low/-cell and the fresh CD34 high+CD38low/-cell isolated from Homo sapiens cord blood in the list by cocultivating again with HESS-5 (secondary culture) The CD34 high+CD38low/-cell was guided also in which cell population. moreover, the property (drawing 48 (it amplifies on HESS-5 previously) --) of the CD34 high+CD38low/-cell amplified and manufactured by each HESS-5 secondary-culture system Drawing 49 (it amplifies on HESS-18 previously) and drawing 50 (it amplifies on HESS-M28 previously) It was the same as that of the property (drawing 51) of the CD34 high+CD38low/-cell which cocultivates with HESS-5 the fresh CD34 high+CD38low/-cell isolated from Homo sapiens cord blood, and is obtained. Moreover, magnification of the CD34 high+CD38low/-cell attained by each HESS-5 secondary-culture system was almost the same as magnification by cocultivation with HESS-5 of a fresh CD34 high+CD38low/-cell isolated from Homo sapiens cord blood also from the viewpoint of the number of proliferating cells, and a reproductive rate so that clearly from Table 6. CD34 positivity cell which cocultivates fresh CD34 positivity cell isolated from Homo sapiens cord blood with a mouse stromata cell strain (HESS-5,

HESS-18, and HESS-M28), and is amplified from this If it is the cell which has the same property as fresh CD34 positivity cell isolated from the Homo sapiens cord blood used as the source and the approach of the invention in this application is used It became clear that the CD34 high+CD38low/-cell which are natural CD34 positivity cell which exists in a Homo sapiens living body, especially a undifferentiated hematopoietic stem cell can be manufactured in large quantities simple.

0073] Analysis of the property of the Homo sapiens CD34 positivity hematopoietic stem cell cultivated and amplified under coexistence of an example 17. mouse stromata cell (2)

For the purpose of the check stated in the example 16, the check from a viewpoint of the cell differentiation ability which this CD34 high+CD38low/-cell has was performed in this experiment. In addition, in this experiment, it analyzed about the differentiation potency to a pre-B cell as an example. Culture of a pre-B cell was performed according to the approach (B.J.Rawlings, Exp.Hematol., 25, and 66 (1991)) reported by Rawlings and others. Each CD34 high+CD38low/-cell isolated in the example 16 namely, the fresh CD34 high+CD38low/-cell isolated from CD34 positivity cell population of (1) Homo-sapiens cord blood origin -- (2) The fresh CD34 high+CD38low/-cell isolated from CD34 positivity cell population obtained from primary cocultivation with HESS-5, (3) The fresh CD34 high+CD38low/-cell isolated from CD34 positivity cell population obtained from primary cocultivation with HESS-18, and the fresh CD34 high+CD38low/-cell, and each 1x 108 piece /, and well isolated from CD34 positivity cell population obtained from primary cocultivation with (4) HESS-M28 -- RPMI-640 culture medium (2ml, made in the Nikken biotechnology medical laboratory.) In addition, 3% fetal calf serum product made from GIBCO-BRL), the 2-mercaptoethanol of 50mM, and recombination Homo sapiens flk-2/flt-3 50 ng(s)/ml ligand (product made from PeproTech EC) are included. In inside, it cocultivated with mouse stromata cell strain HESS-5 (contact culture). Each cells were collected after culture, it dyed by FITC fluorescein isothiocyanate) indicator anti-CD19 monoclonal antibody and PE (phycoerythrin) indicator anti-CD10 monoclonal antibody, and the content of the pre-B cell generated by culture was analyzed using flow cytometer FACSort. In addition, it is one of the indexes of being a pre-B cell that it is CD19 positivity CD10 positivity. The result was shown in drawing 51 thru/or drawing 54 . Furthermore, the result obtained by this flow cytometry was evaluated, and the number of cells and the computed reproductive rate were shown in Table 7.

0074]

Table 7]

表7

HESS-5との二次共培養に供したCD34^{high+}CD38$^{low/-}$細胞	全細胞の増幅率（倍）	B前駆細胞の含有率（%）
ヒト臍帯血から単離した新鮮なCD34^{high+}CD38$^{low/-}$細胞	114.6 ± 8.6	85.1 ± 5.9
各種ストローマ細胞との一次共培養により増幅された細胞群から単離したCD34^{high+}CD38$^{low/-}$細胞		
HESS-5との一次共培養で増幅されたCD34^{high+}CD38$^{low/-}$細胞	101.7 ± 16.3	82.3 ± 3.3
HESS-18との一次共培養で増幅されたCD34^{high+}CD38$^{low/-}$細胞	122.0 ± 17.0	76.7 ± 6.5
HESS-M28との一次共培養で増幅されたCD34^{high+}CD38$^{low/-}$細胞	127.3 ± 22.3	85.0 ± 2.3

＊値は、3回の実験の平均値±SD。

[0075] Also in which cell population, the pre-B cell was guided by cocultivating again each CD34 high+CD38low/-cell which set in the example 5, and was amplified and manufactured by cocultivation (primary culture) with each of HESS-5, HESS-18, and HESS-M28, and the fresh CD34 high+CD38low/-cell isolated from Homo sapiens cord blood in the list with HESS-5 (secondary culture) so that clearly from this drawing. Moreover, the property (drawing 52 (it amplifies on HESS-5 previously), drawing 53 (it amplifies on HESS-18 previously), and drawing 54 (it amplifies on HESS-M28 previously)) of the pre-B cell amplified and manufactured by each HESS-5 secondary-culture system was the same as the property (drawing 51) of the pre-B cell which cocultivates with HESS-5 the fresh CD34 high+CD38low/-cell isolated from Homo sapiens cord blood, and is obtained. Moreover, the property of the differentiation to the pre-B cell attained by each HESS-5 secondary-culture system was almost the same as the result in the secondary culture using HESS-5 of a fresh CD34 high+CD38low/-cell isolated from Homo sapiens cord blood also from the viewpoint of the number of proliferating cells, and a reproductive rate so that clearly from Table 7. CD34 positivity cell which cocultivates fresh CD34 positivity cell isolated from Homo sapiens cord blood with a mouse stromata cell strain (HESS-5, HESS-18, and HESS-M28), and is amplified from this If it is the cell which has the same property as fresh CD34

ositivity cell isolated from the Homo sapiens cord blood used as the source and the approach of the invention n this application is used It became clear that the CD34 high+CD38low/-cell which are natural CD34 positivity ell which exists in the Homo sapiens living body which has the pluripotency which can specialize to a pre-B cell tc., especially a undifferentiated hematopoietic stem cell can be manufactured in large quantities simple.
0076] The cell culture instrument for manufacturing CD34 hematopoietic stem cell by the approach of the nanufacture invention in this application of an example 18. cell culture instrument was designed and created imple on a medical site and/or industry. In addition, the instrument indicated below is a mere example and it annot be overemphasized that it is not that by which the instrument of the invention in this application is imited to this example. The instrument shown in this example has a configuration which is illustrated by drawing 3 , and each element (member) has the following descriptions.
1) the 1st film <quality-of-the-material> polyethylene terephthalate <aperture> 0.4-0.45-micrometer < hole -- bout [number >] -- holes / the 10cm [12cm by / 10cm2 <magnitude>] <thickness> 12-micron (2) 2nd film - and Si CULTURE BAG (the product made from Tissue Culture Supplies &Consumables --) which is the bag of he 3rd film <the quality of the material> commercial item USA; 10cm by [10cm/ 12cm by / insoluble frame quality-of-the-material> rigid plastic <thickness> about 1.5 millimeter <outer diameter> / 10cm / 12cm by / ilm <magnitude> / <property> carbon-dioxide permeability (3) / <bore>] 8cm <frame width> around of the same uality of the material as Wako Pure Chem Agency : 1cm (4) Tubing (arranged between the 1st film and the 2nd ilm and between the 1st film and the 3rd film)
<quality-of-the-material> silicon tube <a bore> -- about 2-3 millimeters <die length> -- about 16cm. The part fixed to the part of the plastic frame of the above (3) (about 1cm.) Not projecting inside is more desirable than a frame. And it consists of about 14cm which comes out of the bag of the above (2). At the end outside of a bag) of a tube, the adapter of the lure lock type which can unstop and close was arranged.
5) The 1st film (1) of construction of an instrument was made to stick by pressure firmly so that it may not separate according to a conventional method in a part for the frame part of the four way type of a plastic frame 3) (it is possible to also make it paste up with still more nearly insoluble adhesives). Subsequently, you made it firmly stuck by pressure so that it may not separate in a part for the frame part of the four way types of the top face of this plastic frame, and an inferior surface of tongue and neither culture medium nor a cell may leak the 2nd film and 3rd film besides an instrument to it according to a conventional method (it is possible to also make t paste up with still more nearly insoluble adhesives). In addition, on the occasion of sticking by pressure of the 2nd film to the plastic frame which stuck this 1st film, the silicon tube (4) was attached in one side of a frame at coincidence. Even if it faced sticking by pressure of the 3rd film, this CHIBU was attached similarly. thus, it has two electric shielding systems in the interior, and the culture bag of the two-layer structure which arranged two tubes which can discharge them from impregnation and this system for air, the liquid, and the cell on the system inside of a bag) of each this was produced. This silicon tube is led to the electric shielding system formed between the 1st film and the 2nd film. a Myelocult H5100 nutrition culture medium (30ml and 12.5% horse blood serum --) The above-mentioned various mouse stromata cell strains made to suspend in the product made from alphaMEM culture-medium;Stem Cell Technologies Inc. which contains fetal calf serum and the 2-mercaptoethanol of 10-4M 12.5% (HESS-5 [for example,]) -- pouring in -- this -- this stromata cell was pasted up on the 1st film. Subsequently, air was removed from the electric shielding system through this tube. The Homo sapiens cord blood origin CD34 positivity cell made to suspend in the Myelocult H5100 nutrition culture medium which, on the other hand, contains recombination Homo sapiens IL-3 and recombination Homo sapiens SCF through a silicon tube in the electric shielding system formed between the 1st film and the 3rd film was poured in. Subsequently, air was removed from the electric shielding system through this tube. A culture bag s put, it cultivated and CD34 positivity hematopoietic stem cell was made to amplify within a CO2 incubator.
0077]
Effect of the Invention] since output is restricted, cord blood is difficult to transplant only for an individual 40kg or less -- etc. -- in order for cord blood stem cell transplantation to be actually able to replace a bone marrow transplantation now, there were many points which should be solved. However, if the approach of this invention is used, since it is possible to manufacture a lot of Homo sapiens CD34 positivity stem cells (especially CD34 high+CD38low/-cell) simple by short culture, it can transplant also to the adult patient of arbitration upwards, and the continuation administration covering several times is attained. Once it extracts cord blood, when required, only a complement can supply a hematopoietic stem cell. Moreover, according to this invention, in a bone marrow transplantation, a lot of bone marrow cells are not needed, but a burden to a donor's mind and body, a problem of safety, etc. accompanying extraction of bone marrow can be avoided. Moreover, while being

ible to treat by extraction of a necessary minimum hematopoietic stem cell by carrying out self-multiplication of he hematopoietic stem cell to diseases, such as neoplastic diseases including acute leukemia, serious illness mmune disorder, congenital enzyme unusual **, and aplastic anemia The cord blood discarded all over the world vith childbirth is utilized effectively, and since transplantation which a transplantation antigen suits by holding he hematopoietic stem cell which has a transplantation antigen with wide width of face is enabled, the risk of erious illness acute graft versus host disease (GVHD) is extremely mitigable. While efficient preservation is ossible if this invention is used when saving a cord blood stem cell, a more efficient hematopoietic stem cell ank can be built by registering the blood which led to a sharp reduction of conservative quantity, and was xtracted once. Furthermore, CD34 positivity cell which has the transplantation antigen which is the need, and vhich is needed by the way can be quickly used by performing mothballs, such as cryopreservation, for the ncreased cell. If the cell culture instrument of this invention is used, furthermore, a desired cell (specifically CD34 positivity cell) and the 2nd desired cell (specifically stromata cell) are heterozoic mutually, or (for example, vhen a stromata cell is a cell of the Homo sapiens origin of CD34 positivity cell in the cell of the mouse origin) Since it can perform separating and refining a mutual cell very easily even if it is the cell extracted from the lifferent individual, transplantation of a Homo sapiens CD34 positivity cell can be quickly carried out to nsurance.

[0078]

[Translation done.]

NOTICES *

.This document has been translated by computer. So the translation may not reflect the original precisely.
.**** shows the word which can not be translated.
.In the drawings, any words are not translated.

)ESCRIPTION OF DRAWINGS

Brief Description of the Drawings]
Drawing 1] Drawing having shown the process which specializes and matures from the hematopoietic stem cell oluripotency hematopoietic stem cell) into the corpuscle cell in blood (a T cell, a B cell, an erythrocyte, a latelet, eosinophile leucocyte, monocyte, neutrophil leucocyte, basophilic leucocyte).
Drawing 2] Drawing having shown how a stromata cell and CD34 positivity cell contact directly in a culture nedium (culture medium), and proliferate CD34 positivity cell a .
Drawing 3] Drawing having shown how to proliferate CD34 positivity cell in the condition that separate a tromata cell and CD34 positivity cell by the film, and the mutual cell does not touch directly. (a) is the case where the number of supporting lamellas is one, (b) is the case where a supporting lamella is two-layer, (c) is in he condition which supported the supporting lamella with support, and (d) shows the condition of having upported the supporting lamella by susceptor.
Drawing 4] That specifically separate CD34 positivity cell and a stromata cell by the film, and a mutual cell is assed and mixed is a desired cell, the 2nd cell, and drawing having shown how to cultivate in the condition that here is nothing. It is the case where the number of supporting lamellas is one, and (b) is the case where a upporting lamella is two-layer, and one supporting lamella and a desired cell are in the condition which eparated, and (c) is the case where a supporting lamella is two-layer, and (a) shows the condition that the lesired cell touches both supporting lamellas.
Drawing 5] Drawing having shown distribution of the corpuscle cell by SSC and FSC of CD34 positivity cell eparated from cord blood.
Drawing 6] Drawing having shown distribution of the corpuscle cell by FITC indicator anti-CD34 antibody of D34 positivity cell and PE indicator anti-CD33 antibody which were separated from cord blood.
Drawing 7] Drawing having shown distribution of the corpuscle cell by SSC and FSC after cultivating for ten lays under existence of cytokine (rh-IL -3 and rh-SCF) under the nonexistence of a stromata cell.
Drawing 8] Drawing having shown distribution of the corpuscle cell by FITC indicator anti-CD34 antibody after ultivating for ten days under existence of cytokine (rh-IL -3 and rh-SCF) under the nonexistence of a stromata ell, and PE indicator anti-CD33 antibody.
Drawing 9] Drawing having shown the number of whole blood spherocytes increased under existence of hydro ortisone or nonexistence and existence of cytokine, or nonexistence.
Drawing 10] Drawing having shown the number of 34 or so CD positivity cells increased under existence of ydro cortisone or nonexistence and existence of cytokine, or nonexistence.
Drawing 11] Drawing having shown the increment scale factor of the number of whole blood spherocytes by the difference in the number of injection cells under existence of rh-SCF and rh-IL -3, existence of HESS-5, or onexistence.
Drawing 12] Drawing having shown the increment scale factor of the 34 or so HESS-CD positivity cell by the difference in the number of injection cells under existence of 5 or nonexistence under existence of rh-SCF and h-IL -3.
Drawing 13] Drawing having shown the increment in the colony count of HPP-CFC (diameter of 2.5mm or more) y cultivating under existence of a stromata cell or nonexistence.
Drawing 14] Drawing having shown the increment in the colony count of HPP-CFC (diameter of 1.0mm or more ess than 2.5mm) by cultivating under existence of a stromata cell or nonexistence.
Drawing 15] Drawing having shown the increment in the colony count of CFU-GM by cultivating under existence of a stromata cell or nonexistence.

[Drawing 16] Drawing having shown the increment in the colony count of an erythroblast system which consists of BFU-E by cultivating under existence of a stromata cell or nonexistence, and a CFU-GEMM colony.
[Drawing 17] Drawing having shown distribution of the corpuscle cell by SSC and FSC after cultivating for ten days under existence of cytokine (rh-IL -3 and rh-SCF) under existence of a stromata cell (HESS-5).
[Drawing 18] Drawing having shown distribution of the corpuscle cell by FITC indicator anti-CD34 antibody after cultivating for ten days under existence of cytokine (rh-IL -3 and rh-SCF) under existence of a stromata cell HESS-5), and PE indicator anti-CD33 antibody.
[Drawing 19] Drawing having shown distribution of the corpuscle cell by SSC and FSC after cultivating for ten days under the nonexistence of cytokine under existence of a stromata cell (HESS-5).
[Drawing 20] Drawing having shown distribution of the corpuscle cell by FITC indicator anti-CD34 antibody after cultivating for ten days under the nonexistence of cytokine under existence of a stromata cell (HESS-5), and PE indicator anti-CD33 antibody.
[Drawing 21] Drawing having shown distribution of the corpuscle cell by SSC and FSC of CD34 positivity cell separated from cord blood.
[Drawing 22] Drawing having shown distribution of the corpuscle cell by SSC and FSC of CD34 positivity cell re-separated from the cell which carried out in vitro culture, and which was increased under existence of cytokine rh-IL -3 and rh-SCF) under existence of a stromata cell (HESS-5).
[Drawing 23] Drawing having shown distribution of CD34 positivity cell by anti-CD33 antibody which carried out PE indicator to anti-CD34 antibody in which CD34 positivity cell separated from cord blood carried out the FITC indicator.
[Drawing 24] Drawing having shown distribution of CD34 positivity cell by FITC indicator anti-CD34 antibody of CD34 positivity cell and PE indicator anti-CD33 antibody which were re-separated from the cell which carried out in vitro culture, and which was increased under existence of cytokine (rh-IL -3 and rh-SCF) under existence of a stromata cell (HESS-5).
[Drawing 25] Drawing having shown distribution of CD34 positivity cell by FITC indicator anti-CD34 antibody of CD34 positivity cell and PE indicator anti-CD38 antibody which were separated from cord blood.
[Drawing 26] Drawing having shown distribution of CD34 positivity cell by anti-CD38 antibody which carried out PE indicator to anti-CD34 antibody in which CD34 positivity cell which carried out in vitro culture under existence of cytokine (rh-IL -3 and rh-SCF) under existence of a stromata cell (HESS-5), and which was re-separated from the increased cell carried out the FITC indicator.
[Drawing 27] Drawing having shown distribution of CD34 positivity cell by FITC indicator anti-CD34 antibody of CD34 positivity cell and PE indicator anti-CD13 antibody which were separated from cord blood.
[Drawing 28] Drawing having shown distribution of CD34 positivity cell by FITC indicator anti-CD34 antibody of CD34 positivity cell and PE indicator anti-CD13 antibody which were re-separated from the cell which carried out in vitro culture, and which was increased under existence of cytokine (rh-IL -3 and rh-SCF) under existence of a stromata cell (HESS-5).
[Drawing 29] Drawing having shown the cell cycle of CD34 positivity cell separated from cord blood.
[Drawing 30] Drawing having shown the cell cycle of a re-dissociating CD34 positivity cell after cultivating for ten days under existence of cytokine (rh-IL -3 and rh-SCF) under existence of a stromata cell (HESS-5).
[Drawing 31] Drawing having made paste up a stromata cell on the film and having shown adhesion or the increment in the whole blood spherocyte under the culture approach according CD34 positivity cell to making it un-paste up on the film.
[Drawing 32] Drawing having made paste up a stromata cell on the film and having shown adhesion or the increment in 34 or so CD positivity cell under the culture approach according CD34 positivity cell to making it un-paste up on the film.
[Drawing 33] Drawing showing the configuration of the instrument for cultivating a cell in instantiation.
[Drawing 34] Drawing showing the property of the cell population containing a Homo sapiens cord blood origin CD34 positivity cell. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to the total cell number of the cell distributed in this field.
[Drawing 35] Drawing showing the property of all the hematopoietic stem cell groups that cultivate a Homo

sapiens cord blood origin CD34 positivity cell under the nonexistence of a stromata cell, and are obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to the total cell number of the cell distributed in this field.

[Drawing 36] Drawing showing the property of all the hematopoietic stem cell groups that cultivate a Homo sapiens cord blood origin CD34 positivity cell under coexistence of stromata cell strain HESS-5, and are obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to the total cell number of the cell distributed in this field.

[Drawing 37] Drawing showing the property of all the hematopoietic stem cell groups that cultivate a Homo sapiens cord blood origin CD34 positivity cell under coexistence of stromata cell strain HESS-18, and are obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to the total cell number of the cell distributed in this field.

[Drawing 38] Drawing showing the property of all the hematopoietic stem cell groups that cultivate a Homo sapiens cord blood origin CD34 positivity cell under coexistence of stromata cell strain HENS-M12, and are obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to the total cell number of the cell distributed in this field.

[Drawing 39] Drawing showing the property of all the hematopoietic stem cell groups that cultivate a Homo sapiens cord blood origin CD34 positivity cell under coexistence of stromata cell strain HESS-M28, and are obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to the total cell number of the cell distributed in this field.

[Drawing 40] Drawing showing the property of CD34 positivity cell of having isolated the Homo sapiens cord blood origin CD34 positivity cell from the hematopoietic stem cell group cultivated and obtained under coexistence of stromata cell strain HESS-5. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to the total cell number of the cell distributed in this field.

[Drawing 41] Drawing showing the property of CD34 positivity cell of having isolated the Homo sapiens cord blood origin CD34 positivity cell from the hematopoietic stem cell group cultivated and obtained under coexistence of stromata cell strain HESS-18. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to

he total cell number of the cell distributed in this field.
Drawing 42] Drawing showing the property of CD34 positivity cell of having isolated the Homo sapiens cord ılood origin CD34 positivity cell from the hematopoietic stem cell group cultivated and obtained under :oexistence of stromata cell strain HESS-M28. A part Fig. (a) shows the gestalt and cell density of a cell, an ıxis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part ig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to he total cell number of the cell distributed in this field.
Drawing 43] Drawing showing the property of the CD34 high+CD38low/-cell population isolated from the Homo ıapiens cord blood origin CD34 positivity cell population. A part Fig. (a) shows the gestalt and cell density of a ell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A ıart Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an ıxis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation :ondition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the ıercentage to the total cell number of the cell distributed in this field.
Drawing 44] Drawing showing the property of the CD34 high+CD38low/-cell population isolated from CD34 ıositivity cell population which cultivated the Homo sapiens cord blood origin CD34 positivity cell under :oexistence of stromata cell strain HESS-5, and was obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a ell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the nanifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field hows the percentage to the total cell number of the cell distributed in this field.
Drawing 45] Drawing showing the property of the CD34 high+CD38low/-cell population isolated from CD34 ıositivity cell population which cultivated the Homo sapiens cord blood origin CD34 positivity cell under :oexistence of stromata cell strain HESS-18, and was obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a ell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the nanifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field hows the percentage to the total cell number of the cell distributed in this field.
Drawing 46] Drawing showing the property of the CD34 high+CD38low/-cell population isolated from CD34 ıositivity cell population which cultivated the Homo sapiens cord blood origin CD34 positivity cell under :oexistence of stromata cell strain HESS-M28, and was obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the nagnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows :he manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the ield shows the percentage to the total cell number of the cell distributed in this field.
Drawing 47] Drawing showing the property of CD34 positivity hematopoietic stem cell which cocultivated the CD34 high+CD38low/-cell population isolated from the Homo sapiens cord blood origin CD34 positivity cell ıopulation with mouse stromata cell HESS-5, and was obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the field shows the percentage to the total cell number of the cell distributed in this field.
[Drawing 48] Drawing showing the property of CD34 positivity hematopoietic stem cell which cultivated further the CD34 high+CD38low/-cell population isolated from CD34 positivity cell population which cultivated the Homo sapiens cord blood origin CD34 positivity cell under coexistence of stromata cell strain HESS-5, and was obtained under coexistence of stromata cell strain HESS-5, and was obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell-surface-differentiation antigens

CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows he manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the ield shows the percentage to the total cell number of the cell distributed in this field.

Drawing 49] Drawing showing the property of CD34 positivity hematopoietic stem cell which cultivated further he CD34 high+CD38low/−cell population isolated from CD34 positivity cell population which cultivated the lomo sapiens cord blood origin CD34 positivity cell under coexistence of stromata cell strain HESS−18, and was btained under coexistence of stromata cell strain HESS−5, and was obtained. A part Fig. (a) shows the gestalt nd cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the nagnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell−surface−differentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an axis of abscissa shows he manifestation condition of CD34. The quadrisected numeric value which was respectively indicated to the ield shows the percentage to the total cell number of the cell distributed in this field.

Drawing 50] Drawing showing the property of CD34 positivity hematopoietic stem cell which cultivated further he CD34 high+CD38low/−cell population isolated from CD34 positivity cell population which cultivated the lomo sapiens cord blood origin CD34 positivity cell under coexistence of stromata cell strain HESS−M28, and vas obtained under coexistence of stromata cell strain HESS−5, and was obtained. A part Fig. (a) shows the estalt and cell density of a cell, an axis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) hows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell−surface− lifferentiation antigens CD34 and CD38, an axis of ordinate shows the manifestation condition of CD38, and an xis of abscissa shows the manifestation condition of CD34. The quadrisected numeric value which was espectively indicated to the field shows the percentage to the total cell number of the cell distributed in this ield.

Drawing 51] Drawing showing the property of the pre−B cell which cocultivated the CD34 high+CD38low/−cell opulation isolated from the Homo sapiens cord blood origin CD34 positivity cell population with mouse stromata ell HESS−5, and was obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an axis of ordinate SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the nanifestation condition of the cell−surface−differentiation antigens CD10 and CD19, an axis of ordinate shows he manifestation condition of CD10, and an axis of abscissa shows the manifestation condition of CD19. The uadrisected numeric value which was respectively indicated to the field shows the percentage to the total cell umber of the cell distributed in this field.

Drawing 52] Drawing showing the property of the pre−B cell which cultivated further the CD34 high+CD38low/− ell population isolated from CD34 positivity cell population which cultivated the Homo sapiens cord blood origin D34 positivity cell under coexistence of stromata cell strain HESS−5, and was obtained under coexistence of tromata cell strain HESS−5, and was obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an xis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part ig. (b) shows the manifestation condition of the cell−surface−differentiation antigens CD10 and CD19, an axis of rdinate shows the manifestation condition of CD10, and an axis of abscissa shows the manifestation condition f CD19. The quadrisected numeric value which was respectively indicated to the field shows the percentage to he total cell number of the cell distributed in this field.

Drawing 53] Drawing showing the property of the pre−B cell which cultivated further the CD34 high+CD38low/− ell population isolated from CD34 positivity cell population which cultivated the Homo sapiens cord blood origin D34 positivity cell under coexistence of stromata cell strain HESS−18, and was obtained under coexistence of tromata cell strain HESS−5, and was obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an xis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part ig. (b) shows the manifestation condition of the cell−surface−differentiation antigens CD10 and CD19, an axis of rdinate shows the manifestation condition of CD10, and an axis of abscissa shows the manifestation condition f CD19. The quadrisected numeric value which was respectively indicated to the field shows the percentage to he total cell number of the cell distributed in this field.

Drawing 54] Drawing showing the property of the pre−B cell which cultivated further the CD34 high+CD38low/− ell population isolated from CD34 positivity cell population which cultivated the Homo sapiens cord blood origin D34 positivity cell under coexistence of stromata cell strain HESS−M28, and was obtained under coexistence f stromata cell strain HESS−5, and was obtained. A part Fig. (a) shows the gestalt and cell density of a cell, an xis of ordinate (SSC) shows cell density, and an axis of abscissa (FSC) shows the magnitude of a cell. A part Fig. (b) shows the manifestation condition of the cell−surface−differentiation antigens CD10 and CD19, an axis of

rdinate shows the manifestation condition of CD10, and an axis of abscissa shows the manifestation condition f CD19. The quadrisected numeric value which was respectively indicated to the field shows the percentage to he total cell number of the cell distributed in this field.

Translation done.]

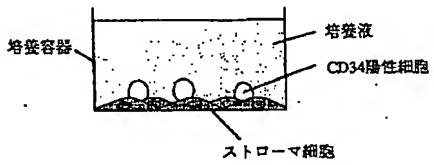
NOTICES *

DRAWINGS

Drawing 2]

図 2

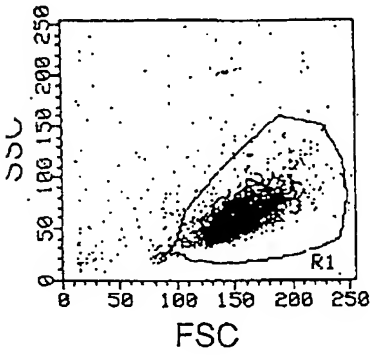
CD34陽性細胞の培養例（接触培養）



Drawing 5]

図 5

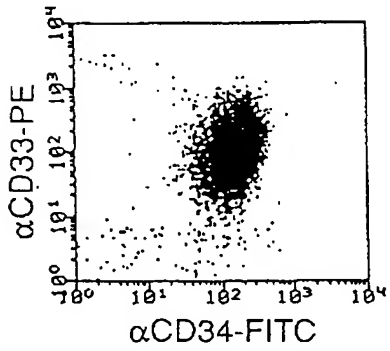
臍帯血由来CD34陽性細胞の分布



Drawing 6]

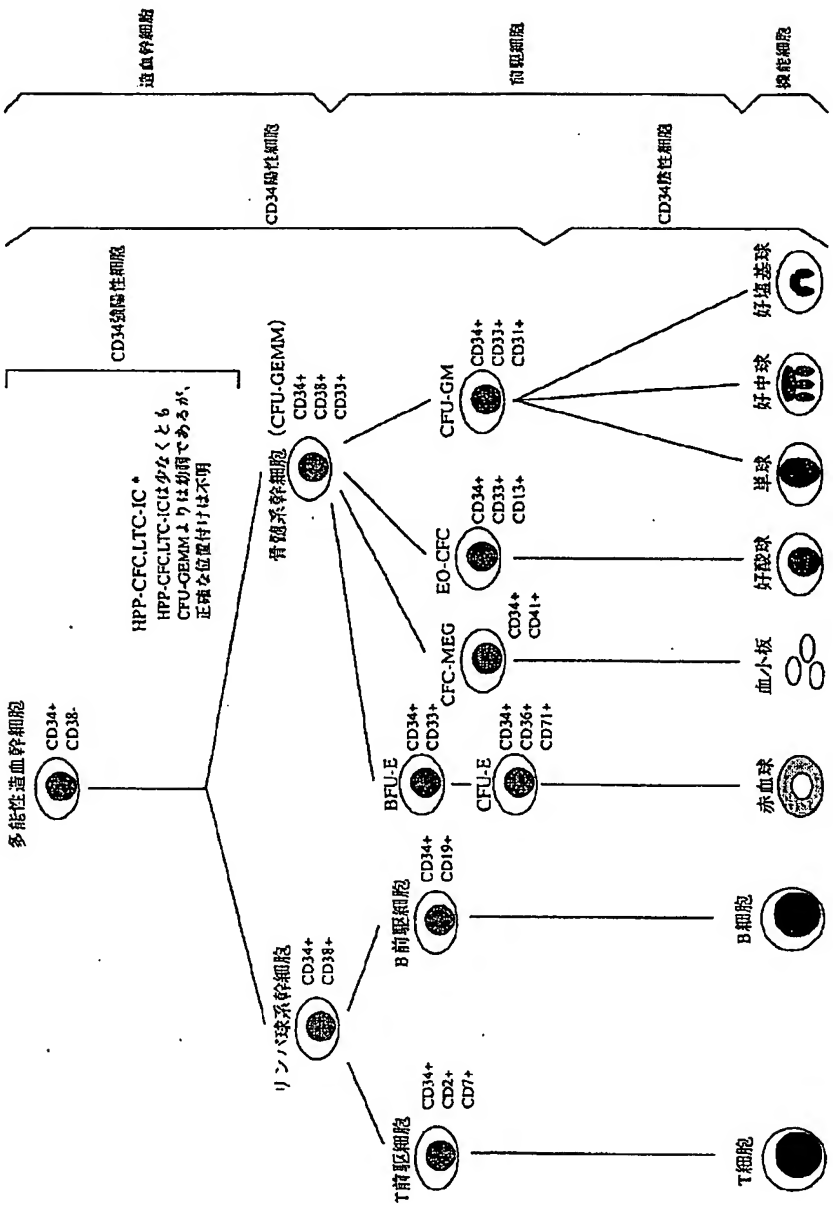
図 6

臍帯血由来CD34陽性細胞の分布



Drawing 1]

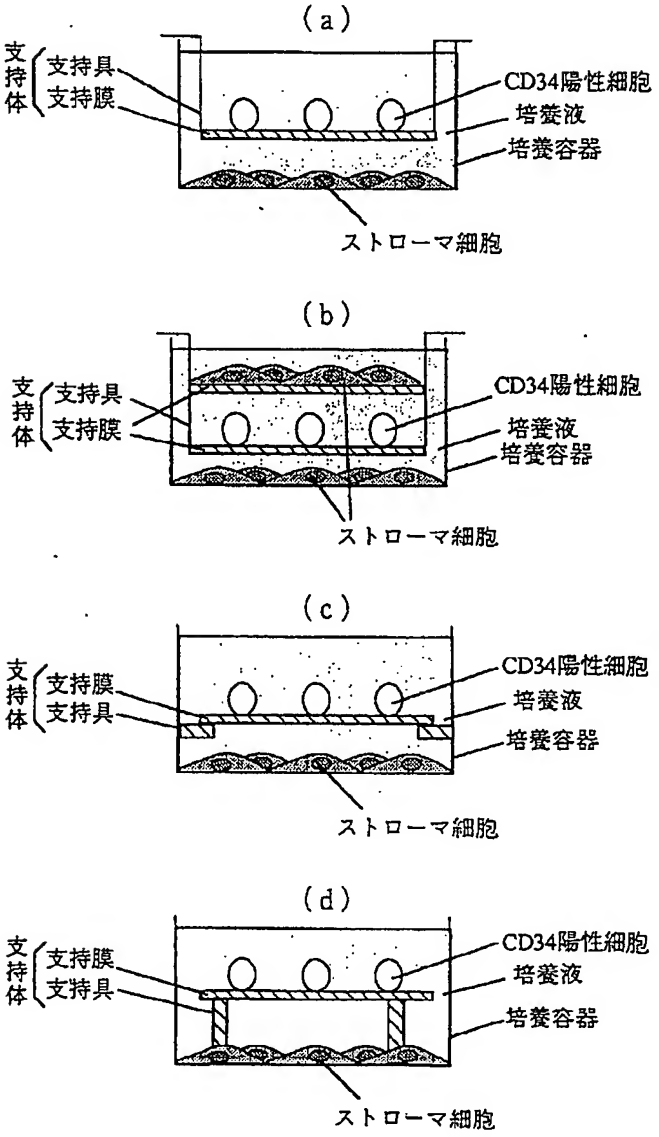
図 1
血液細胞の分化の模式図



Drawing 3]

図 3

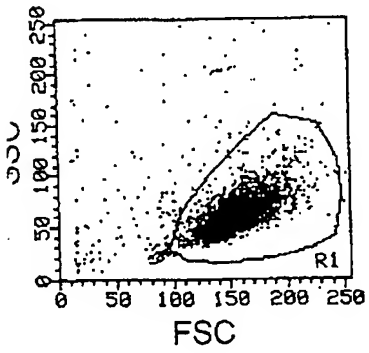
CD34陽性細胞の培養例（非接触培養）



[Drawing 21]

図 21

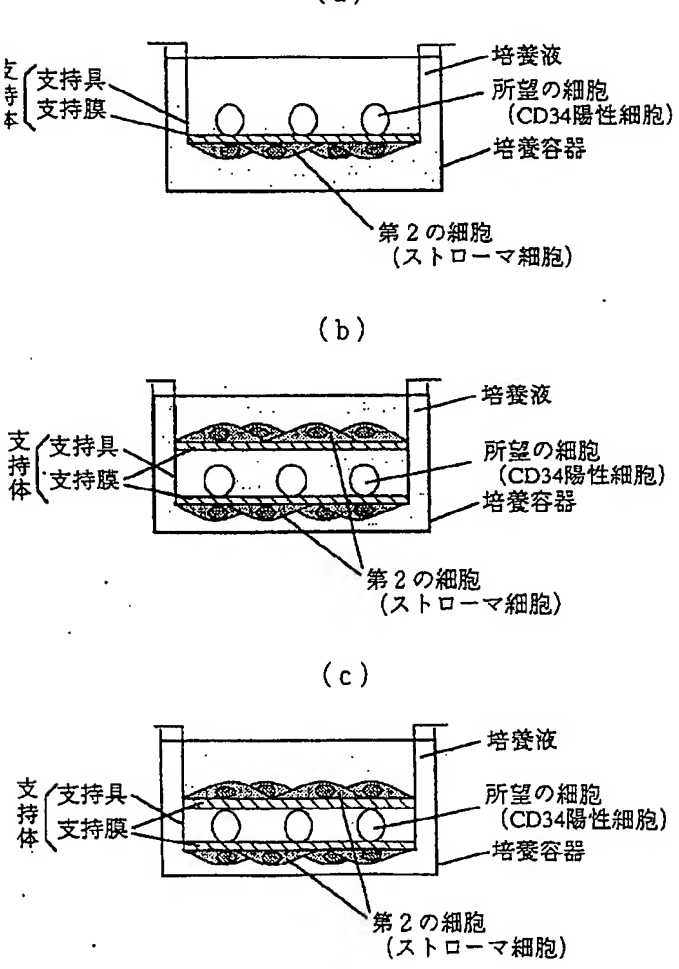
臍帯血由来CD34陽性細胞の分布



Drawing 4]

図 4

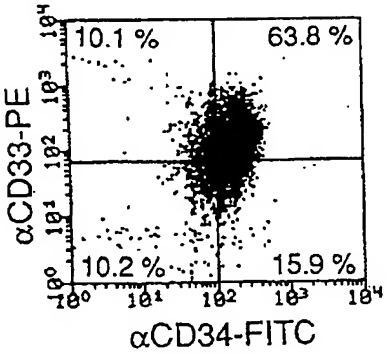
CD34陽性細胞の培養例（間接接触培養）



Drawing 23]

図 23

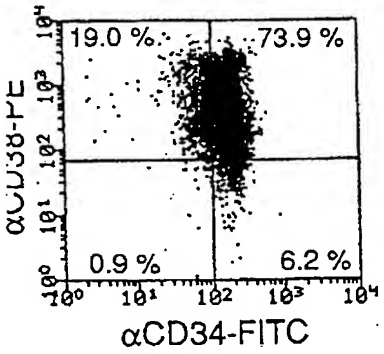
臍帯血由来CD34陽性細胞の分布



Drawing 25]

図 25

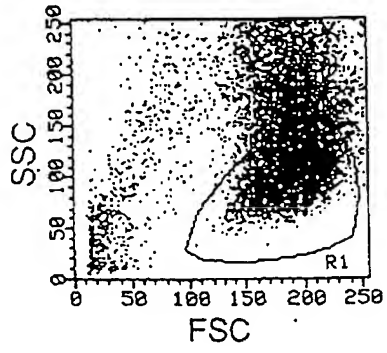
臍帯血由来CD34陽性細胞の分布



Drawing 7]

図 7

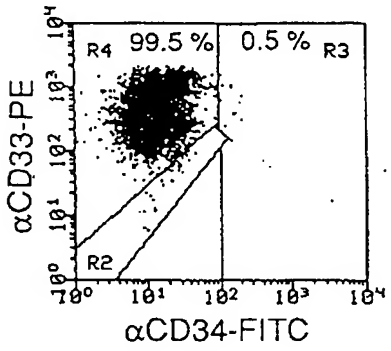
ストローマ細胞の非存在下、サイトカインの存在下での
CD34陽性細胞の分布



Drawing 8]

図 8

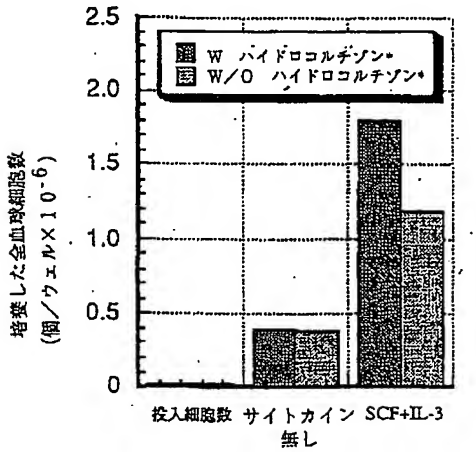
トローマ細胞の非存在下、サイトカインの存在下での
CD34陽性細胞の分布



Drawing 9]

図 9

ハイドロコルチゾン、サイトカインの存在下又は非存在下での
全血球細胞数

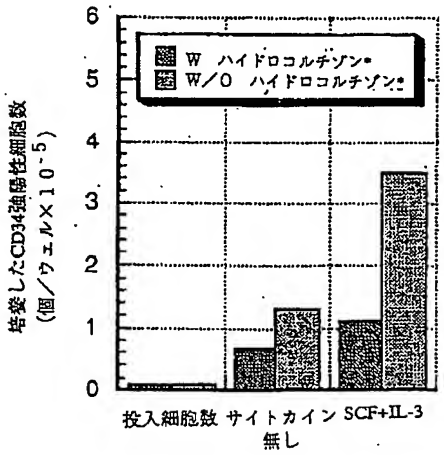


W ハイドロコルチゾンはハイドロコルチゾン存在下、
W/Oハイドロコルチゾンはハイドロコルチゾン非存在
下を意味する

Drawing 10]

図 10

ハイドロコルチゾン、サイトカインの存在下又は非存在下での
CD34陽性細胞数

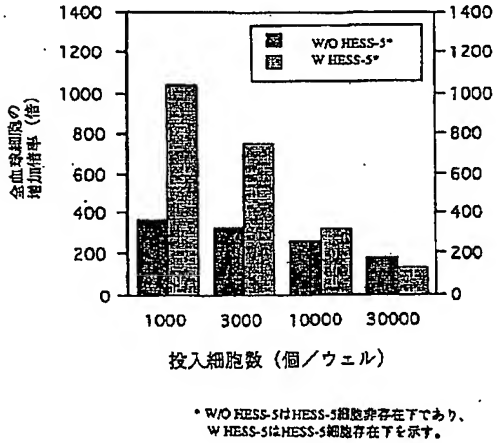


* W ハイドロコルチゾンはハイドロコルチゾン存在下、
W/Oハイドロコルチゾンはハイドロコルチゾン非存在
下を意味する

[Drawing 11]

図 11

サイトカイン存在下におけるHESS-5細胞の存在下又は非存在下での
全血球細胞の増加倍率

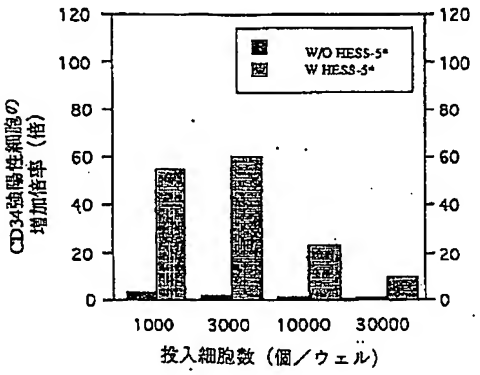


* W/O HESS-5はHESS-5細胞非存在下であり、
W HESS-5はHESS-5細胞存在下を示す。

[Drawing 12]

図 12

トイトカイン存在下におけるHESS-5細胞の存在下又は非存在下での
CD34陽性細胞の増加倍率

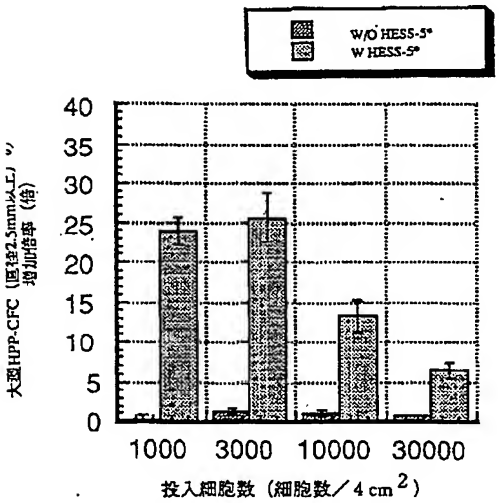


* W/O HESS-5はHESS-5細胞非存在下であり、
W HESS-5はHESS-5細胞存在下を示す。

[Drawing 13]

図 13

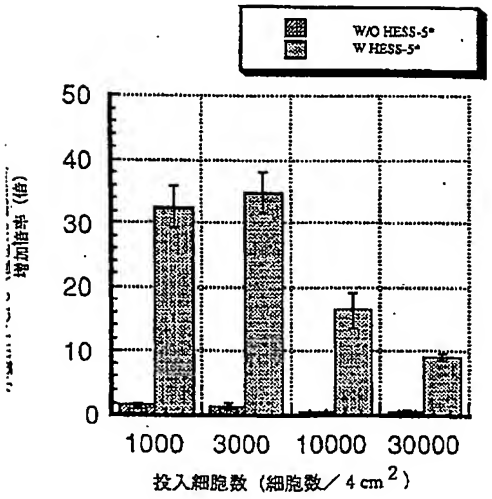
HESS-5細胞の存在下又は非存在下での
大型HPP-CFCコロニーの増加倍率



[Drawing 14]

図 14

HESS-5細胞の存在下又は非存在下での
小型HPP-CFCコロニーの増加倍率

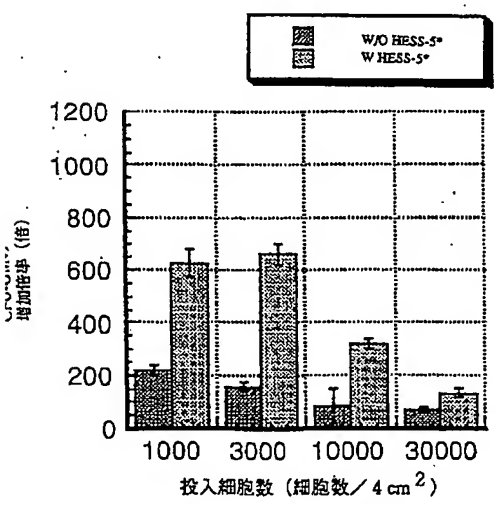


* W/O HESS-5はHESS-5細胞非存在下であり、
W HESS-5はHESS-5細胞存在下を示す。

Drawing 15]

図 15

HESS-5細胞の存在下又は非存在下での
CFU-GMコロニーの増加倍率

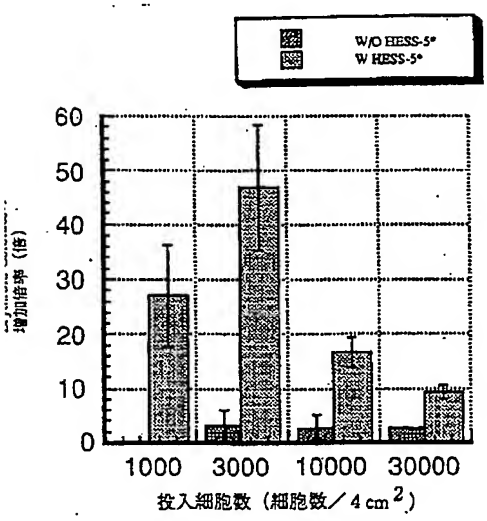


* W/O HESS-5はHESS-5細胞非存在下であり、
W HESS-5はHESS-5細胞存在下を示す。

Drawing 16]

図 16

HESS-5細胞の存在下又は非存在下での
赤血球系細胞コロニーの増加倍率

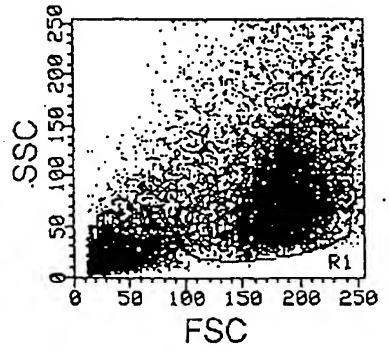


* W/O HESS-5はHESS-5細胞非存在下であり、
W HESS-5はHESS-5細胞存在下を示す。

[Drawing 17]

図 17

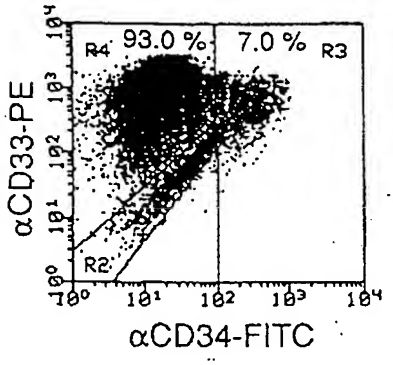
ストローマ細胞の存在下、サイトカインの存在下での
血液細胞の分布



[Drawing 18]

図 18

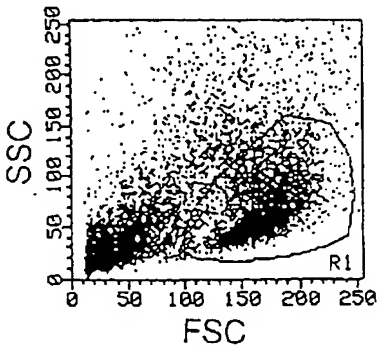
ストローマ細胞の存在下、サイトカインの存在下での
血液細胞の分布



Drawing 19]

図 19

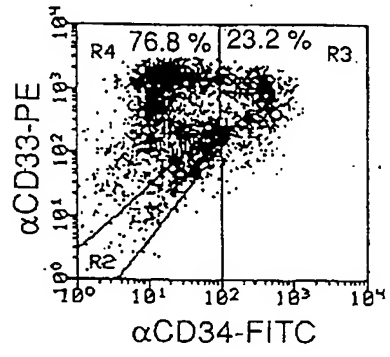
ストローマ細胞の存在下、サイトカインの非存在下での
血液細胞の分布



Drawing 20]

図 20

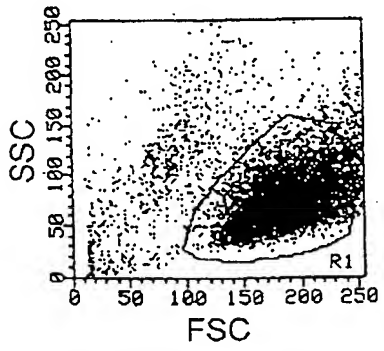
ストローマ細胞の存在下、サイトカインの非存在下での
血液細胞の分布



[Drawing 22]

図 22

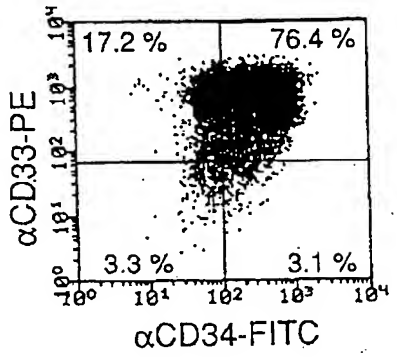
ESS-5細胞の存在下、サイトカインの存在下で再分離した
血液細胞の分布



Drawing 24]

図 24

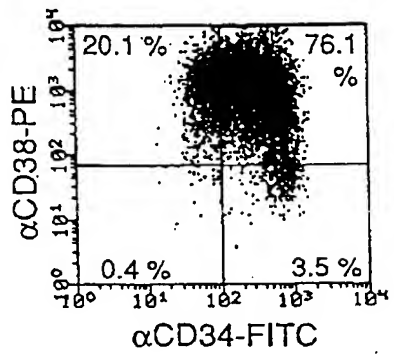
IESS-5細胞の存在下、サイトカインの存在下で再分離した
血液細胞の分布



Drawing 26]

図 26

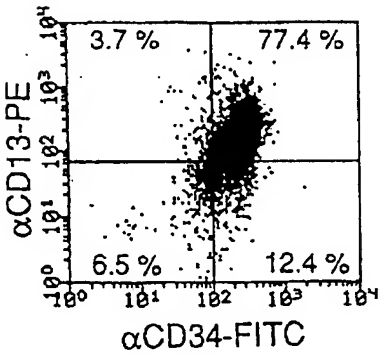
IESS-5細胞の存在下、サイトカインの存在下で再分離した
血液細胞の分布



Drawing 27]

図 27

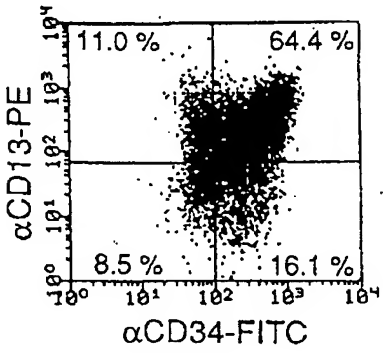
臍帯血由来CD34陽性細胞の分布



Drawing 28]

図 28

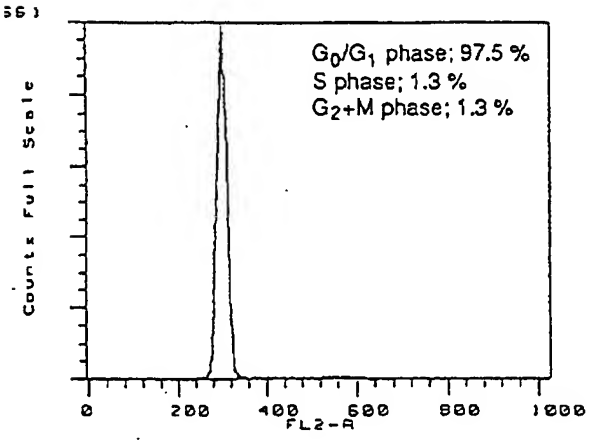
ESS-5細胞の存在下、サイトカインの存在下で再分離した
血液細胞の分布



Drawing 29]

図　２９

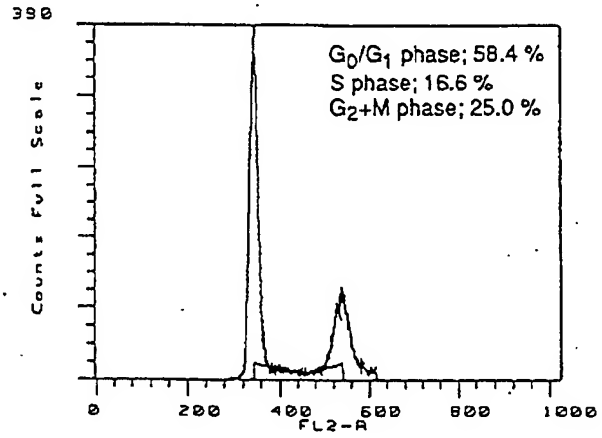
臍帯血由来CD34陽性細胞の細胞周期



Drawing 30]

図　３０

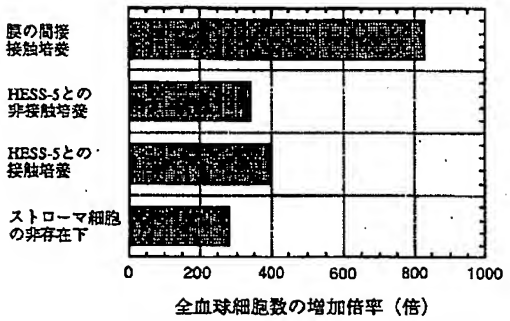
HESS-5細胞の存在下、サイトカインの存在下で再分離した
CD34陽性細胞の細胞周期



Drawing 31]

図 31

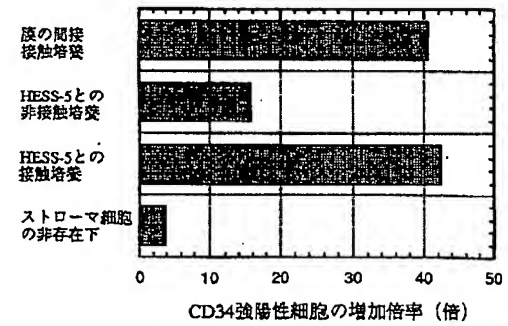
]接接触培養法、非接触培養法、接触培養法及びストローマ細胞の非存在下での全血球細胞の増加倍率



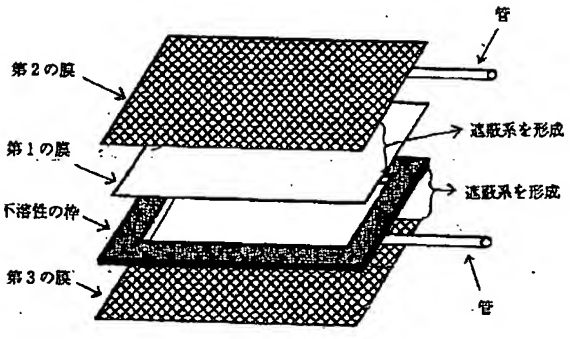
Drawing 32]

図 32

]接接触培養法、非接触培養法、接触培養法及びストローマ細胞の非存在下でのCD34強陽性細胞の増加倍率

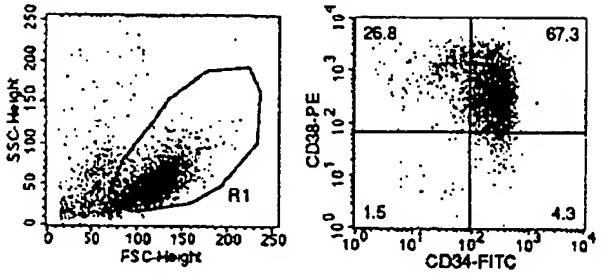


Drawing 33]



[Drawing 34]

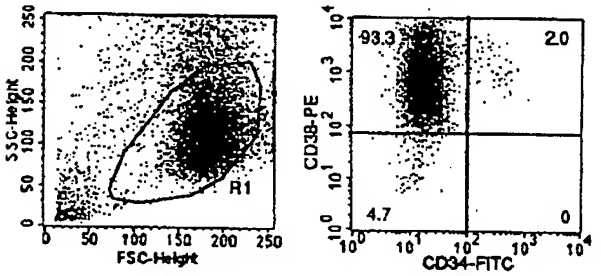
ヒト臍帯血から取得した
新鮮なCD34陽性細胞



(a) (b)

Drawing 35]

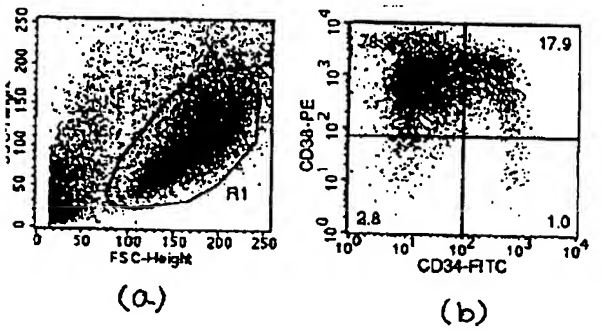
ストローマなし



(a) (b)

Drawing 36]

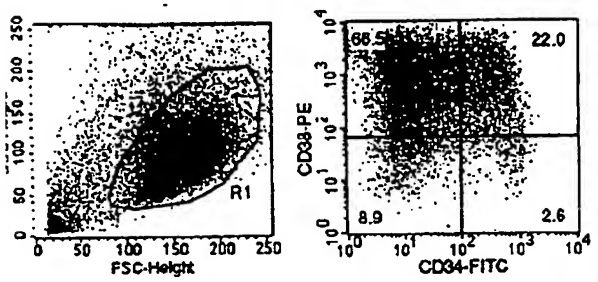
HESS-5 cells



(a) (b)

Drawing 37]

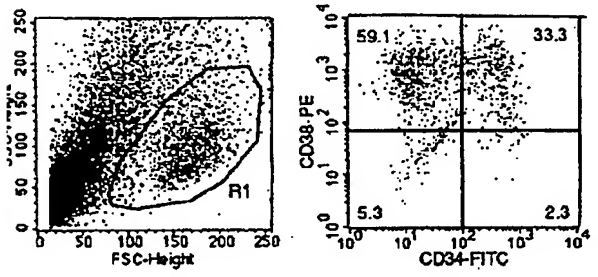
HESS-18 cells



(a) (b)

[Drawing 38]

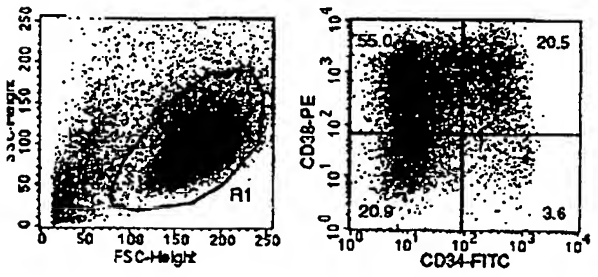
HENS-M12 cells



(a) (b)

[Drawing 39]

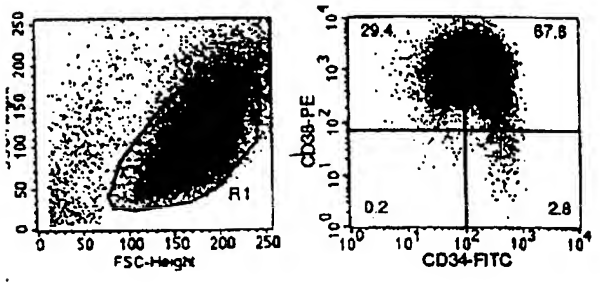
HESS-M28 cells



(a) (b)

[Drawing 40]

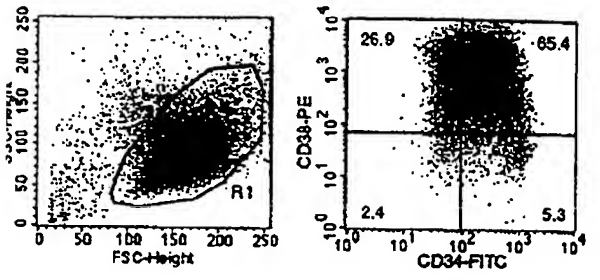
HESS-5 cells



(a) (b)

[Drawing 41]

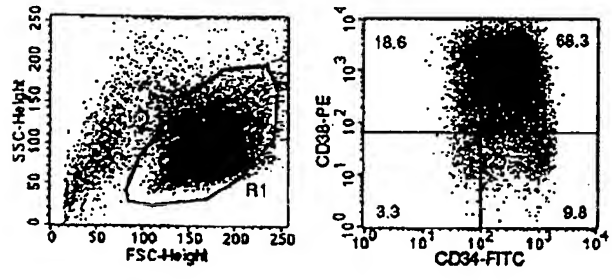
HESS-18 cells



(a) (b)

[Drawing 42]

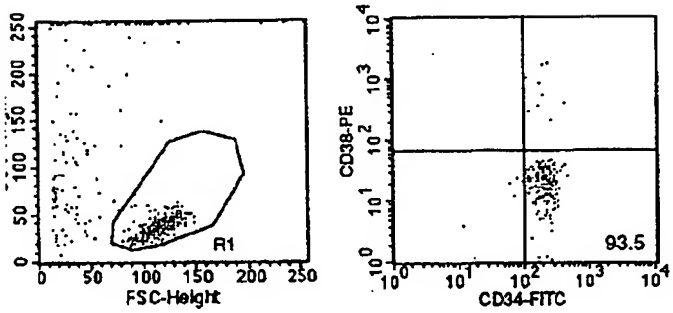
HESS-M28 cells



(a) (b)

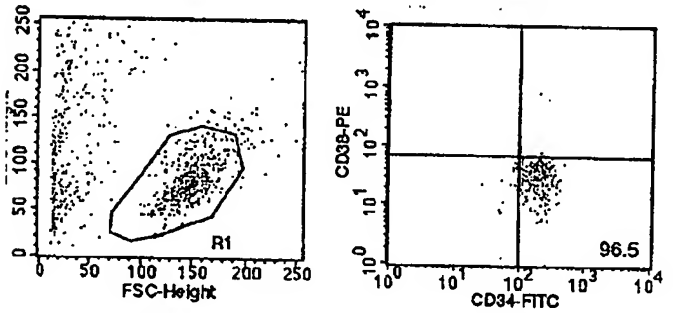
[Drawing 43]

ヒト臍帯血から取得した
新鮮なCD34^{high+}CD38$^{low/-}$細胞



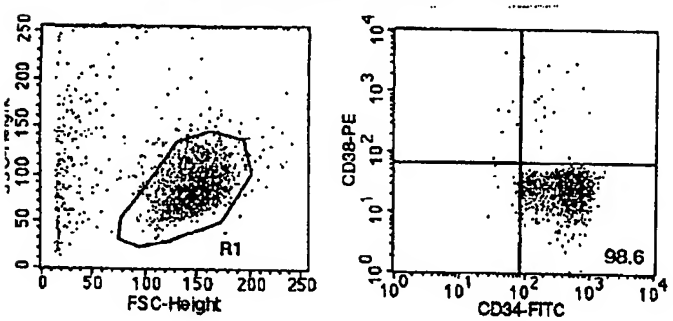
Drawing 44]

HESS-5との一次共培養系から
単離したCD34^{high+}CD38$^{low/-}$細胞



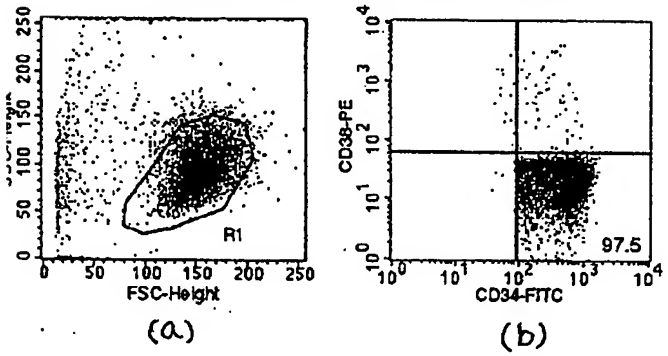
Drawing 45]

HESS-18との一次共培養系から
単離したCD34^{high+}CD38$^{low/-}$細胞



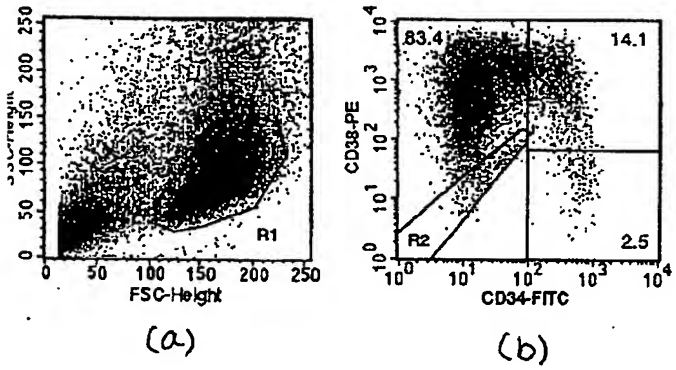
Drawing 46]

HESS-M28との一次共培養系から
単離したCD34^{high+}CD38$^{low/-}$細胞



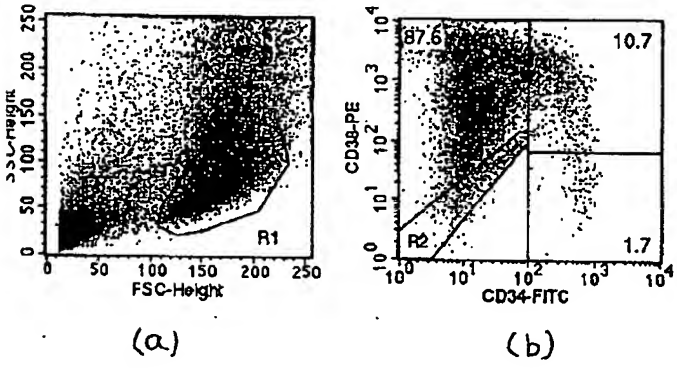
[Drawing 47]

ヒト臍帯血から取得した
新鮮なCD34^{high+}CD38$^{low/-}$細胞



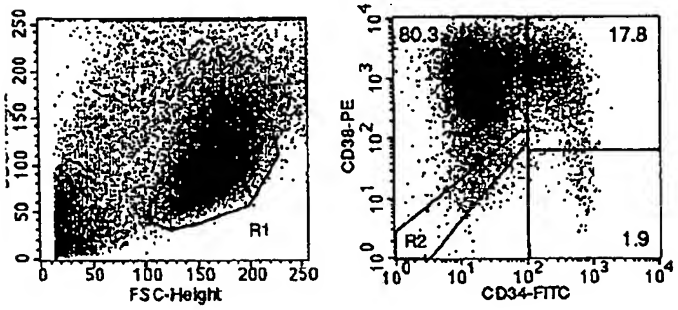
[Drawing 48]

HESS-5との一次共培養系から
単離したCD34^{high+}CD38$^{low/-}$細胞



[Drawing 49]

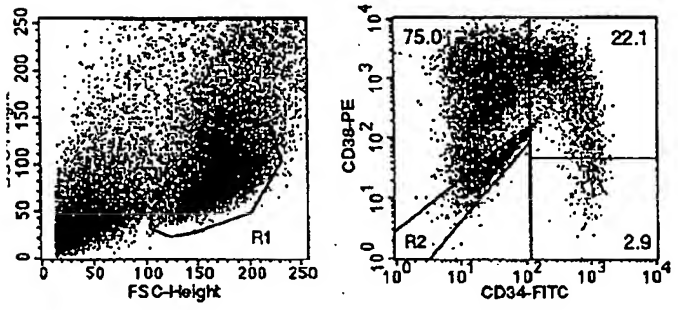
HESS-18との一次共培養系から
単離したCD34^{high+}CD38$^{low/-}$細胞



(a) (b)

Drawing 50]

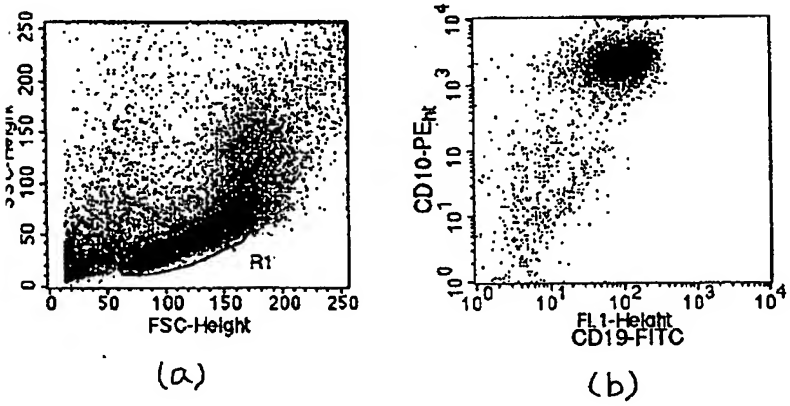
HESS-M28との一次共培養系から
単離したCD34^{high+}CD38$^{low/-}$細胞



(a) (b)

Drawing 51]

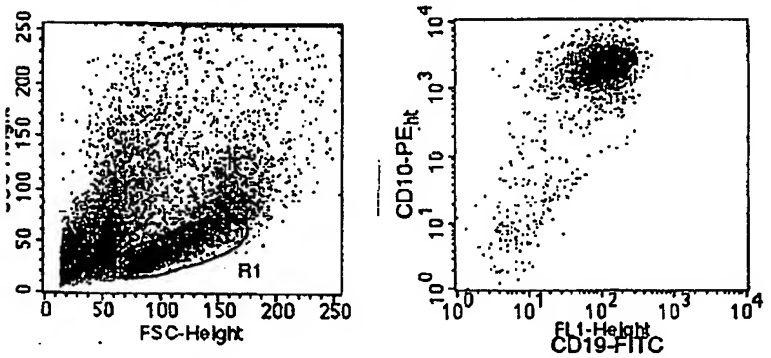
ヒト臍帯血から単離した
新鮮なCD34陽性細胞



(a) (b)

Drawing 52]

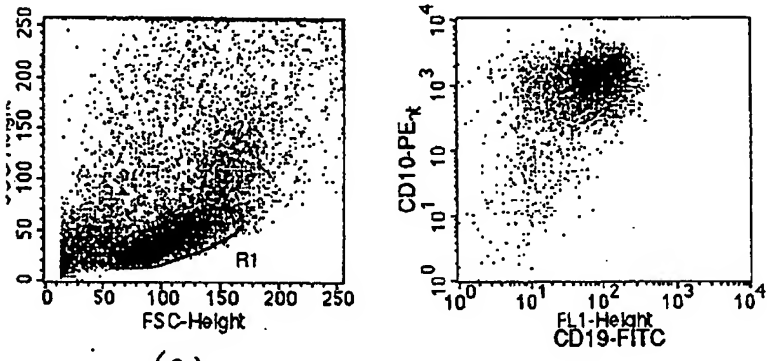
HESS-5との一次共培養系から
単離したCD34^{high+}CD38$^{low/-}$細胞



(a) (b)

Drawing 53]

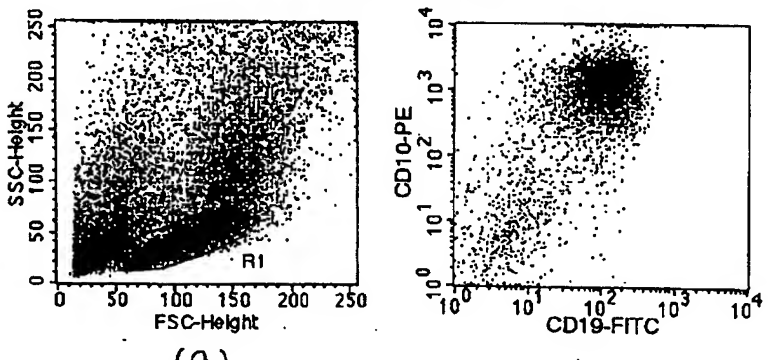
HESS-18との一次共培養系から
単離したCD34^{high+}CD38$^{low/-}$細胞



(a) (b)

Drawing 54]

HESS-M28との一次共培養系から
単離したCD34^{high+}CD38$^{low/-}$細胞



(a) (b)

Translation done.]

he case where the 2nd cell (specifically stromata cell) is heterozoic mutually, and the case of an animal of the ame kind, It is desirable to separate a stromata cell and CD34 positivity cell and to use only CD34 positivity cell or transplantation. If the 2nd or 3rd culture approach is used, even when a stromata cell and CD34 positivity ell are cells of the Homo sapiens origin of CD34 positivity cell mutually in the case of an another solid-state or eterozoic (for example, the cell of the mouse origin of a stromata cell), separation and purification of a mutual ell can carry out very easily, and transplantation of the hematopoietic stem cell which is CD34 positivity cell an carry out to insurance quickly.

0042] It can set to cultivate also about culture [which / of the 1st, 2nd, and 3rd culture approaches], and can arry out in a suitable culture container. A stromata cell can maintain and survive, and maintenance, survival, ifferentiation and maturation, although self-renewal is carried out, as long as CD34 positivity cell does not heck a culture container at all, it may use the thing of what kind of material and a configuration. As a material f a culture container, glass, synthetic resin, natural resin, a metal, etc. are specifically mentioned, and multiple pindles, such as multiple columns, such as the triangle pole, a cube, and a rectangular parallelepiped, a triangular yramid, and a square drill, the configuration of arbitration like a gourd, a globular form, a semi-sphere, circular, n ellipse form, a hemicycle, etc. are specifically mentioned as a configuration. Culture may be under an open ondition and may be under a closing (sealing) condition. About culture medium (culture medium), a stromata cell an maintain and survive, and maintenance, survival, differentiation and maturation, although self-renewal is arried out, if CD34 positivity cell does not prevent at all, what kind of culture medium (culture medium) can be sed. Maintenance, survival, differentiation and maturation, although self-renewal is carried out, as long as it is in harge of cultivating, a stromata cell can maintain and survive as chemical environment conditions, such as hysical environment conditions, such as temperature, osmotic pressure, and light, oxygen, carbon dioxide gas, H, and an oxidation reduction potential, and CD34 positivity cell does not prevent at all, you may be what kind f environmental condition. About temperature, it is specifically 30 degrees C thru/or 40 degrees C, and is 37 legrees C preferably. It is specifically osmotic pressure [in / osmotic pressure / physiology conditions], and is lesirable osmotic pressure equal to a physiological saline. As a light, you may be the conditions as a dark room, nd it may be as bright as the brightness of the outside at the time of fine weather. The dissolved oxygen oncentration in the condition that the oxygen density in a gaseous phase specifically touches [the culture ystem] 10% of gaseous phase as an oxygen density thru/or the oxygen density in a gaseous phase may be xygen densities in the condition of being in contact with 30% of gaseous phase, and it is an oxygen density in he condition of being in contact with the gaseous phase of the dissolved oxygen concentration in the condition hat the oxygen density in a gaseous phase touches 20% of gaseous phase preferably. It is specifically pH6.0 hru/or pH8.0 as pH for generally controlling pH in a culture system, and is desirable pH equivalent to physiology onditions. In order to control pH, a carbon dioxide may be used, and what kind of other buffer solutions may be sed. It is the dissolved carbon-dioxide-gas concentration in the condition that the culture system specifically ouches 5% of gaseous phase as concentration of carbon dioxide gas.

0043] Furthermore, in the culture medium in a culture container (culture medium), other chemical entities or a iogenic substance like a blood serum can also be contained according to inorganic substances, such as sodium, potassium, calcium, magnesium, Lynn, and chlorine, amino acid, a vitamin, hormone, an antibiotic, cytokine, a atty acid, sugar, or the purpose. The usual approach can be used when saving a stromata cell, CD34 positivity ell (preferably CD34 positivity cell of the Homo sapiens origin) extracted from mammalian, or CD34 positivity ell cultivated and increased (it contains for a long period of time also). What is necessary is to mention for xample, a cryopreservation method as the approach of preservation, to add freezing defense agents, such as a lycerol, ethylene glycol, dimethyl sulfoxide (DMSO), sucrose, a glucose, and a polyvinyl pyrrolidone (PVP), if eeded in this case, to perform slow speed freezing using a program freezer etc., and just to save in liquid itrogen etc. after that.

0044]

Example]

he cytokine used by the preparation (1) Homo-sapiens cytokine and monoclonal antibody this example of an xample 1. ingredient It is the thing of marketing which was obtained according to the purification method or was btained by the modifying-gene method. Specifically A stem cell factor (rh-SCF), a granulocyte colony-timulating factor (rh-G-CSF), flk2/flt3 ligand (rh-flk2L), macrophage inflammatory protein 1alpha (rh-MIP-alpha), Solubilization interleukin -6 acceptor (rh-sIL-6R), interleukin 3 (rh-IL -3, product made from Genzyme), hey are a granulocyte-monocyte colony stimulating factor (rh-GM-CSF, product made from Genzyme), nterleukin -6 (rh-IL -6), the refined ERUSUROPO ethyne (EPO, Connaught Laboratories), etc. moreover, about

he monoclonal antibody (mAbs) used for the cell surface marker analysis by flow cytometry in this example
\nti-CD34 antibody (clone HPCA-2) which carried out the fluorescein isothiocyanate (FITC) indicator is
urchased from Beckton Dickinson Imunocytometry System (product made from San Lose). R-(phycoerythrin
E) indicator anti-CD33 antibody (clone WM-15), PE-indicator anti-CD13 antibody (clone WM-15), PE-indicator
nti-CD33 antibody (clone WM-53), and PE-indicator anti-CD38 antibody (clone HIT2) were purchased from
harminngenn.
0045] (2) stromata cell strain hematogenous support ability -- having (operation which helps the hematogenous
unctions of a hematopoietic stem cell) -- the stromata cell strain and the stromata cell strain which does not
ave hematogenous support ability were established from mouse bone marrow and a spleen. About the existence
f hematogenous support ability, it examined by Dexter- and Whitlock-Witte-type long term culture out of the
iving body. The mouse origin stromata cell strain which has the hematogenous support ability used in this
nvention is HESS-5, HESS-1, HESS-18, HESS-M28, SSXL CL.3, and SSXLCL.7. It was SSXL CL.9 and SSXL
CL.17 cell. Moreover, HENS-M28 was prepared as a stromata cell without hematogenous support ability. These
ell strains were cultivated in alpha-minimal essential MEDIUMU (alpha-minimal essential mediumu; alpha-MEM;
he Nikken biotechnology medical laboratory company make) which added 10% (V/V) horse blood serum (HS;
nade in the Nichimen United States) of carbon dioxides 5% at 37 degrees C.
0046] Extraction of CD34 positivity cell from example 2. Homo sapiens cord blood and preparation Homo
apiens cord blood were extracted in the Tokyo monopoly hospital obstetrics and gynecology, after performing
ufficient informed consent for a donor, and they were used under the guideline established in the Tokyo
nonopoly hospital and the Japan Tobacco, Inc. physic fundamental research laboratories. The umbilical cord was
itigated by the two-place clamp by the part near a newborn infant after giving birth to a newborn infant, and
etween clamps was cut horizontally. Suction extraction was carried out with the syringe from the upper part of
he ligature-of-cord part by the side of a placenta, and it extracted in the test tube which added heparin so that
t might be set to 20 units / ml. In this case, cord blood of 50 to 120 ml was extractable from one placenta. After
xtracting a blood sample from a donor, it was saved at 4 degrees C and used within 48 hours. According to the
lensity gradient centrifugation for which the cord blood sample used phosphorus FOPUREPPU (Lymphoprep,
roduct made from Nycomed Pharma AS), specific gravity extracted the 1.077g [/ml] or less low-specific-
gravity cell. Next, they are CD34 pro JIENITA isolation kit (product made from Progenitor Isolation Kit
QBend/10)), and a MACS-magnetic cel about CD34 positivity cell and CD34 shade sexual cell. Sorting system
according to the directions-for-use manual and the alpha MEDDO press (Alpha Med Press, pp.201-213), it
lissociated using Magnetic Cell Sorting System (product made from Miltenyi Biotec GmbH).)
0047] It is the 2ml MIERO cult H5100 () in each well of 12 well tissue culture plate (product made from Falcon)
bout the decision CD34 positivity cell (3x103 cell / ml) of the field of the 34 or so example 3. cord blood CD
ositivity cell. [12.5% horse blood serum (HS)] And 12.5% new-born calf serum (FCS), 10-4M The alpha-
MEM:STEMCELL Technologies Inc . company make reinforced by 2-mercaptoethanol is added. 20 ng/ml rh-IL-
t cultivated under existence of ng of 3 and 50/ml rh-SCF. Ten days after cultivating, pipetting was fully
erformed, cells were collected, by the nylon mesh, after filtration, centrifugal separation (for 1000g, 4 degrees
C, and 5 minutes) was carried out, and CD34 positivity cells were collected. The obtained cell performed immuno
ull ORESSENSU dyeing, and measured the cell surface marker by flow cytometry (FACSort).
0048] The experiment about the property of the cord blood origin CD34 positivity cell under example 4. flow-
ytometry measurement **** and stromata cell nonexistence etc. was conducted. Flow-cytometry analysis of
D34 positivity cell was performed according to the following procedures. The phosphate buffer solution (PBS-)
vhich does not contain calcium2+ which added cow serum albumin (BSA) and 5mM EDTA for the cell collected
ccording to the aforementioned centrifugal separation 0.5 more%, and Mg2+ was re-distributed, and it dyed by
nti-CD34 antibody and anti-CD33 antibody which carried out PE indicator which carried out the FITC indicator.
After leaving it for 30 minutes in Hikami, the cell was washed 3 times with the same buffer solution as the
bove-mentioned, and this buffer solution was re-distributed. The dyed cell measured the property distribution
ver the relation between the magnitude/density distribution of the cell, and CD34 antibody / CD33 antibody
sing the fluorescence metering device FACSort (product made from Becton DickinsonImmunocytometry
Systems). The result is shown in drawing 5 thru/or drawing 8 . drawing showing the relation between Forward
catter (the magnitude of a FSC:CD34 positivity cell is meant) of CD34 positivity cell which separated drawing 5
rom cord blood, and Side scatter (SSC: this consistency) -- it is -- drawing 6 -- and it is shown, respectively,
he fluorescence intensity of manifestations, i.e., amount, of FITC and PE when dyeing by FITC indicator anti-
D34 antibody (alphaCD34) and PE indicator anti-CD33 antibody (clone WM-15). According to drawing 5 , it